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(54) Title: MODIFIED BIOLOGICAL MATERIAL (57) Abstract Conventionally, animal tissue from one species can only be transplanted into another species when the species are concordant; otherwise, hyperimmune rejection ensues. In this invention, donor tissue is modified, for example by being transgenic, to express or otherwise be in association with one or more substances, referred to as homologous complement restriction factors (HCRFs), which are active in the recipient species to prevent the complete activation of complement and therefore rejection. The invention is in part based on the discovery that the alternative pathway of complement activation, rather than the classical pathway, is responsible for hyperacute discordant xenograft rejection.		

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MODIFIED BIOLOGICAL MATERIAL

This invention relates to biologically compatible material for use in transplants, and to the production and use of such material.

The replacement of failed or faulty animal (particularly human) tissue, including organs, has over the last four decades become a common place therapy in clinical medicine. These replacement therapies range for example from the use of the polyethylene terephthalate sold under the trade mark DACRON by DuPont to repair faulty blood vessels to the use of saphenous vein as an autograft to by-pass blocked arteries and to the transplantation from one human to another of a heart.

Organ transplantation has undergone significant development with modern immunosuppressants allowing high success rates to be achieved at relatively modest cost. The demand for organ transplantation has increased rapidly. There are now more than 20,000 organ transplants per annum carried out worldwide. This, however, represents only approximately 15% of the need as assessed by current criteria. The supply/demand ratio of donor organs of all types can not be met from existing sources. This is perhaps best illustrated with the demand for heart transplantation. The first heart transplantation by Barnard in 1967 generated considerable press coverage. Within a year, 101 heart transplants had been performed in 22 countries by 64 different surgical teams. Disillusionment followed the poor results obtained so

1 that by the early 1970s fewer than 30 transplants per
2 year were being performed worldwide. The introduction
3 of cyclosporin immuno-suppression, however, has
4 revolutionarised heart transplantation so that most
5 centres can now anticipate success rates for heart
6 transplantation of more than 80% one year graft (and
7 patient survival). As expertise is gained, this
8 survival rate can reasonably be expected to increase
9 further. The success of this procedure, of course,
10 fuels demand so that the medical profession and the
11 general public become more aware that heart
12 transplantation offers a real alternative to death, so
13 more and more patients are referred for the procedure.
14 Currently, over 2,000 heart transplants per annum are
15 performed.

16
17 Today, the greatest risk of death in heart
18 transplantation is while waiting for a suitable donor
19 organ to become available. While the artificial heart
20 offers a short-term support device for these patients,
21 long-term demands are for more heart transplant centres
22 and a greater donor supply. The potential number of
23 individuals who might benefit from cardiac
24 transplantation has never been scientifically
25 established, but published estimates of the need for
26 heart transplantation have ranged widely between 50 and
27 250 people per million per year depending on selection
28 criteria, age of recipient, disease and so forth.
29 Whatever the actual figure may be, it is quite clear
30 already that current donor supply options are incapable
31 of meeting demand. Similar comments can be made for
32 kidney and liver transplantation, and it seems likely
33 that once pancreas or Islet of Langerhans cell

1 transplantation becomes a widely-accepted therapeutic
2 procedure for the treatment of diabetes, shortage of
3 this tissue will also become a prime concern.
4

5 There are further disadvantages with current
6 transplantation therapy. It is by no means always the
7 case that donor organs are fit for use in
8 transplantation, not least because many organ donors
9 are themselves victims of some accident (for example, a
10 road accident) which has caused death by injury to some
11 organ other than that which is being transplanted;
12 however, there may be some additional injury to or
13 associated difficulty with the organ to be
14 transplanted.
15

16 Further, because of the unpredictable availability of
17 organs from donors, transplant surgery often can not be
18 scheduled as a routine operation involving theatre time
19 booked some while in advance. All too frequently,
20 surgical teams and hospital administrators have to
21 react the moment a donor organ is identified and work
22 unsocial hours, thereby adding to administrative and
23 personal difficulties.
24

25 In the case of heart, liver and lung transplants, if
26 rejection is encountered it will not usually be
27 possible to retransplant unless by chance another
28 suitable donor becomes available within a short space
29 of time.
30

31 Apart from the above medical difficulties, current
32 transplantation practice can in some cases involve
33 social difficulties. In the first place, there may be

1 religious objections to removing organs from potential
2 donors, particularly in cultures believing in
3 reincarnation. There are of course other ethical and
4 social difficulties encountered in removing organs from
5 dead humans, particularly as consent is required in
6 some countries. Finally, the appearance of a
7 commercial trade in live kidney donors is causing
8 concern, particularly in certain third world countries,
9 and it would be socially desirable to suppress or
10 reduce such a trade.

11

12 Conventional transplantation surgery, as outlined above
13 with its disadvantages, involves the transplantation
14 from one animal of a particular species (generally
15 human) to another of the same species. Such
16 transplantations are termed allografts. Because of the
17 difficulties with conventional allograft supply, as
18 outlined above, attention has focused on the
19 possibility of using xenografts in transplantation.
20 Xenografting is the generic term commonly used for the
21 implantation of tissues, including cells and organs,
22 across species barriers.

23

24 There have already been several examples of the
25 successful use of xenografts in therapeutic replacement
26 schedules. For example, recent years have witnessed
27 the use of pig tissue for aortic valve replacement, pig
28 skin to cover patients with severe burns, and cow
29 umbilical vein as a replacement vein graft. All of
30 these xenografts have, however, one point in common:
31 they provide a mechanical replacement only. The tissue
32 used is biologically non-functional. The reason for
33 this is that the immune processes existing in man

1 immediately (within minutes or hours) destroy the
2 cellular integrity of tissues from most species. Such
3 xenografts are known as discordant xenografts.

4

5 The ferocity of this destruction is phylogenetically
6 associated. Thus, tissue from the chimpanzee, which is
7 a primate closely related to man, can survive in man in
8 much the same way as an allograft; such a xenograft is
9 known as a concordant xenograft.

10

11 While it may be thought that concordant xenografts
12 might provide the answer to the difficulties with
13 allografts, in practice this is probably not the case.
14 Chimpanzees are much smaller than man and chimpanzee
15 organs are generally not big enough to work in man. In
16 the case of kidneys this may be overcome by
17 transplanting two chimpanzee kidneys to replace a
18 failed human kidneys, but for liver and heart this is
19 clearly not a possibility. Furthermore, chimpanzees
20 breed slowly in nature and poorly in captivity, and the
21 demand for chimpanzees as experimental animals
22 (particularly in the current era of research into
23 Acquired Immune Deficiency Syndrome (AIDS)) means that,
24 yet again, demand is outstripping supply.
25 Additionally, there may be some social difficulty with
26 the public acceptance of the use of other primates as
27 xenograft donors.

28

29 Attention has therefore refocused on discordant
30 xenografts. It has been commonly believed that the
31 reason why discordant xenografts fail so rapidly, is
32 the existence in the recipient species of "naturally
33 occurring" antibodies against as yet undefined antigens

1 of the donor species (Shons et al (Europ. Surg. Res. 5
2 26-36 (1973)). The antibodies are called "naturally
3 occurring" because they are found to exist in
4 individuals who have not had any immunological
5 challenge from the donor species.

6
7 The rapid rejection - known as hyperacute antibody-
8 mediated rejection - of an organ graft is well
9 documented. In the early 1960s, when (allograft)
10 kidney transplantation became a routine treatment, it
11 was observed that transplanted kidneys were
12 occasionally rejected by the recipient whilst the
13 operation was still in progress. During a transplant
14 operation, the kidney will as a rule become red and
15 firm in consistency soon after the vessels of the
16 recipient and donor are sutured together. Such
17 transplants often produce urine almost immediately. In
18 the form of rejection where the graft is destroyed
19 while the patient is still on the table (hyperacute
20 rejection) the destructive processes begin in the first
21 few minutes or so after transplantation. When this
22 occurs, the kidney becomes bluish and patchy and then
23 congested. The consistency of the organ is also
24 altered. As a rule, the graft becomes oedematous, no
25 urine production occurs and the newly-transplanted
26 kidney is then immediately removed. It has become
27 clear that a humorally-mediated immunological response
28 between preformed circulating antibodies in the
29 recipient and antigens in the donor kidney are
30 involved. The only way to avoid its occurrence in
31 allografting is to check before transplantation that
32 there are no antibodies existing in the recipient
33 against the donor cells. With increased knowledge of

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1 testing for such antibodies (known as the cross match)
2 it has become clear that this generalisation that
3 antibody in the recipient reacts against antigens in
4 the donor is not true and that hyperacute graft
5 destruction, when it involves transplants between
6 individuals of the same species is restricted to the
7 existence of specific sorts of antibody known as T-warm
8 positive cross-match; and almost certainly these
9 antibodies belong to the IgG subclass. Furthermore,
10 the presence of these antibodies always results from a
11 pre-existing immunisation procedure either as a result
12 of previous blood transfusions or as a result of
13 pregnancy or, most commonly, as a result of a failed
14 previous transplant.

15

16 The mechanism for hyperacute xenograft rejection has
17 largely been thought to be much the same as the
18 mechanism for hyperacute allograft rejection, as
19 outlined above. The literature on the mechanism of
20 xenograft rejection is extensive, stretching back some
21 83 years. During that time, only three publications
22 appear to have suggested a mechanism for xenograft
23 rejection which does not involve antibodies. The
24 suggestion was that the alternative pathway of
25 complement activation was implicated in xenograft
26 rejection (although not necessarily using such
27 terminology). The suggestion first appeared in 1976 in
28 a paper by Schilling et al (Surgery, Gynaecology and
29 Obstetrics 142 29-32 (1976)). The suggestion was made
30 again in 1988 and 1989 (the same data were published
31 twice) by Miyagawa et al (Transplantation 46(6) 825-830
32 (1988) and Transplantation Proceedings 21(1) 520-521
33 (1989)). However, the results were not conclusive,

1 because both these experiments suffered from
2 substantially the same fault. The model chosen is
3 claimed by the authors to be a xenograft model in which
4 cross-species antibodies did not exist. However, it
5 now appears that the assays used to detect
6 cross-species antibodies were inadequate, and that the
7 inferences drawn in these papers were based on
8 inadequate data.

9
10 Most measures currently taken experimentally to avoid
11 or reduce rejection in xenografts involve
12 chemotherapeutically interfering with the recipient's
13 immune system, largely on a non-specific basis for
14 example with cyclosporin A and other
15 immunosuppressants, by plasmaphoreses, by treatment
16 with cobra venom factor, Staphylococcus protein A
17 absorption of antibody and so on. This approach
18 naturally follows from the chemotherapy that supports
19 allografts.

20
21 This invention adopts a radically different approach:
22 instead of non-specifically interfering with the
23 recipient's immune system, the invention enables to co-
24 administration of material which has the effect of the
25 donor graft being regarded as self by certain
26 components of the recipient's immune system. In
27 particularly preferred embodiments, the donor tissue
28 itself is modified to appear immunologically to the
29 recipient to be self in certain respects.

30
31 It is has also been discovered that hyperacute
32 xenograft rejection is not necessarily antibody-
33 mediated. This arises from two observations. First,

1 in the absence of antibody but the presence of
2 complement, hyperacute rejection is observed; secondly,
3 in the presence of antibody but the absence of
4 complement, no hyperacute rejection is observed.

5

6 The invention is based on the discovery that complement
7 activation is pre-eminent in the hyperacute destruction
8 of a xenograft whether or not such activation is aided
9 by the binding of appropriate antibody molecules.
10 Activation of the alternative pathway of complement can
11 be induced by a variety of cell products. These
12 products are not restricted to foreign-invading cells
13 such as bacteria or xenografts but exist on many cells.
14 Thus, in principle, many cells of an individual could
15 activate the alternative pathway of complement, causing
16 massive auto-immune destruction. That this does not
17 happen is due to the existence of a number of
18 complement down-regulating proteins naturally present
19 in serum and on the surface of cells. These molecules
20 (referred to herein as "homologous complement
21 restriction factors") prevent the complete activation
22 of self complement either by the classical or
23 alternative pathway by the products of self cells, thus
24 preventing the auto-immune destruction of self. The
25 functioning of such molecules is elegantly illustrated
26 in paroxysmal nocturnal haemoglobinuria. In this
27 disease, the membrane anchor of at least one of these
28 molecules (decay accelerating factor) is absent. Thus,
29 the protein is not retained in the erythrocyte cell
30 membrane and detaches from the cell, which activates
31 the alternative pathway of complement and is then lysed
32 thus causing haemoglobinuria.

33

1 According to a first aspect of the present invention,
2 there is provided a method of transplanting animal
3 tissue into a recipient, wherein the tissue is derived
4 from a donor of a different species from the recipient,
5 the donor species being a discordant species with
6 respect to the recipient, the method comprising
7 grafting the tissue into the recipient and providing in
8 association with the grafted tissue one or more
9 homologous complement restriction factors active in the
10 recipient species to prevent the complete activation of
11 complement.
12

13 The word "tissue" as used in this specification means
14 any biological material that is capable of being
15 transplanted and includes organs (especially the
16 internal vital organs such the heart, lung, liver and
17 kidney, pancreas and thyroid) cornea, skin, blood
18 vessels and other connective tissue, cells including
19 blood and haematopoietic cells, Islets of Langerhans,
20 brain cells and cells from endocrine and other organs
21 and body fluids (such as PPF), all of which may be
22 candidates for transplantation from one species to
23 another.
24

25 A "discordant species" is a species a (generally
26 vascularised) xenograft from which into the recipient
27 would normally give rise to a hyperacute rejection,
28 that is to say rejection within minutes or hours and
29 not days (Calne Transplant Proc 2:550, 1970). Such
30 hyperacute rejections will be well known to those
31 skilled in the art, and ay take place in under 24
32 hours, under 6 hours or even under one hour after
33 transplantation.

1 Complement and its activation are now well known, and
2 are described in Roitt, Essential Immunology (Fifth
3 Edition, 1984) Blackwell Scientific Publications,
4 Oxford. The activity ascribed to complement (C')
5 depends upon the operation of nine protein components
6 (C1 to C9) acting in sequence, of which the first
7 consists of three major sub-fractions termed C1q, C1r
8 and C1s. Complement can be activated by the classical
9 or alternative pathway, both of which will now be
10 briefly described.

11
12 In the classical pathway, antibody binds to C1, whose
13 C1s subunit acquires esterase activity and brings about
14 the activation and transfer to sites on the membrane or
15 immune complex of first C4 and then C2. This complex
16 has "C3-convertase" activity and splits C3 in solution
17 to produce a small peptide fragment C3a and a residual
18 molecule C3b, which have quite distinct functions. C3a
19 has anaphylatoxin activity and plays no further part in
20 the complement amplification cascade. C3b is membrane
21 bound and can cause immune adherence of the
22 antigen-antibody-C3b complex, so facilitating
23 subsequent phagocytosis.

24
25 In the alternative pathway, the C3 convertase activity
26 is performed by C3bB, whose activation can be triggered
27 by extrinsic agents, in particular microbial
28 polysaccharides such as endotoxin, acting independently
29 of antibody. The convertase is formed by the action of
30 Factor D on a complex of C3b and Factor B. This forms
31 a positive feedback loop, in which the product of C3
32 breakdown (C3b) helps form more of the cleavage enzyme.
33

1 In both the classical and alternative pathways, the C3b
2 level is maintained by the action of a C3b inactivator
3 (Factor I). C3b readily combines with Factor H to form
4 a complex which is broken down by Factor I and loses
5 its haemolytic and immune adherence properties.

6
7 The classical and alternative pathways are common after
8 the C3 stage. C5 is split to give C5a and C5b
9 fragments. C5a has anaphylatoxin activity and gives
10 rise to chemotaxis of polymorphs. C5b binds as a
11 complex with C6 and C7 to form a thermostable site on
12 the membrane which recruits the final components C8 and
13 C9 to generate the membrane attack complex (MAC). This
14 is an annular structure inserted into the membrane and
15 projecting from it, which forms a transmembrane channel
16 fully permeable to electrolytes and water. Due to the
17 high internal colloid osmotic pressure, there is a net
18 influx of sodium ions and water, leading to cell lysis.

19
20 Homologous complement restriction factors (HCRFs)
21 useful in the present invention can in general
22 interfere with any part of the complement activation
23 cascade. An HCRF may interfere solely with that part
24 which constitutes the classical pathway, or solely with
25 that part which constitutes the alternative pathway, or
26 more usually may interfere with that part which is
27 common to both the classical and alternative pathways.
28 It is preferred that the HCRF regulator interfere with
29 the common part of the pathway. The HCRF may be
30 identical to a natural HCRF or simply have the
31 appropriate function. Synthetic and semi-synthetic
32 HCRFs, including those prepared by recombinant DNA
33

1 technology and variants however prepared, are included
2 within the term HCRF.

3

4 As has been mentioned above, homologous complement
5 restriction factors are substances which regulate the
6 action of the complement cascade in such a way as to
7 reduce or prevent its lytic activity; they are used by
8 the animal body to label tissue as self to avoid
9 autoimmune reaction. In this invention it is possible
10 in principle for the HCRF to be either membrane bound
11 or free in serum, although in practice it will be
12 preferred to have the HCRF being membrane bound on
13 cells of the xenograft tissue. In this way, it is
14 easier for the HCRF to be "in association with" the
15 graft tissue. Preferred HCRFs include putative cell
16 membrane factors including the C3b/C4b receptor (CR1),
17 C3dg receptor (CR2), decay accelerating factor (DAF),
18 C3b Inactivator and membrane cofactor protein (MCP).
19 Putative serum HCRFs include Factor H, decay
20 accelerating factor (DAF) and C4 binding protein
21 (C4bp). These HCRFs all down-regulate the activity of
22 complement by interference at the C3 stage. Homologous
23 restriction factor (HRF), which blocks at C8, is also a
24 putative membrane factor.

25

26 Many, but not all, of the genes for suitable HCRFs are
27 located in the RCA (regulator of complement activation)
28 locus, which map to band q32 of chromosome 1
29 (Rey-Campos et al J. Exp. Med. 167 664-669 (1988)).

30

31 Although there has been some confusion with the
32 nomenclature and location of HCRFs, the factors C4BP,
33 CR1, DAF and Factor H are identified by Rey Campus et

1 al (loc. cit.) and in their earlier study (J. Exp. Med.
2 166 246-252 (1987)). Membrane cofactor protein (MCP)
3 is treated by some workers as synonymous with C4
4 binding protein (C4bp) and it may be that these two
5 factors are either related or identical. Rother and
6 Till ("The Complement System", Springer-Verlog, Berlin
7 (1988)) review the regulatory factors of C3 convertase
8 in section 1.2.3.2; they equate C4 binding protein
9 (C4bp) with decay accelerating factor and Factor H with
10 B₁H-protein and C3b Inactivator Accelerator. No doubt
11 the nomenclature, localisation and characterisation of
12 HCRFs will continue to evolve, but it is to be
13 understood that the present invention contemplates the
14 use of all HCRFs as suitability and preference dictate.

15
16 Other references to HCRFs are as follows:

17 Factor I (also previously known as C3b inactivator
18 or KAF):
19 Tamura & Nelson (J. Immunol. 99 582-589 (1967);

20
21 Factor H: Pangburn et al (J. Exp. Med. 146 257-270
22 (1977);

23
24 C4 binding protein: Fujita et al (J. Exp. Med. 148
25 1044-1051 (1978));

26
27 DAF (also known as CD55): Nicholson-Weller et al
28 (J. Immunol. 129 184 (1982));

29
30 Membrane Cofactor Protein (MCP; also known as CD46
31 and first described as gp45-70 and further known
32
33

1 as gp66/56): Seya et al (J. Exp. Med. 163 837-855
2 (1986));

3
4 CR1 (also known as CD35): Medof et al, (J. Exp.
5 Med. 156 1739-1754 (1982)) and Ross et al (J.
6 Immunol. 129 2051-2060 (1982));

7
8 CR2 (also known as CD21, 3d/EBV receptor and
9 p140): Iida et al (J. Exp. Med. 158 1021-1033
10 (1983)) and Weis et al (PNAS 81 881-885 (1984)).

11

12 The tissue distribution of some of the RCA proteins are
13 as follows:

14

15 CR1: Membrane (limited): erythrocytes;
16 monocytes; most B and some T cells;
17 polymorphonuclear leukocytes; follicular-dendritic
18 cells; glomerular podocytes;

19

20 CR2: Membrane (limited): most B cells;
21 follicular-dendritic cells; some epithelial cells
22 and a few T cell lines;

23

24 MCP: Membrane (wide): all peripheral blood
25 cells (but erythrocytes); epithelial, endothelial
26 and fibroblast cell lineages; trophoblast and
27 sperm;

28

29 DAF: Membrane (wide): all peripheral blood
30 cells; epithelial, endothelial and fibroblast cell
31 lineages; trophoblast and sperm;

32

33 C4bp: Plasma: liver synthesis; and

1
2 H: Plasma: liver synthesis; fibroblast and
3 monocyte cell lines.
4

5 As for proteins involved in homologous restriction at
6 the level of the membrane attack complex, the use of
7 which is also contemplated by means of the present
8 invention, there is general agreement (but as yet no
9 proof) in the form of a protein sequence that the
10 following 65kDa (or thereabouts) proteins are
11 identical:

12
13 C8 binding proteins (Schönermark et al, J.
14 Immunol. 136 1772 (1986));

15
16 homologous restriction factor (HRF) (Zalman et al
17 Immunology 83 6975 (1986)); and

18
19 MAC-inhibiting protein (MIP) (Watts et al.
20 (1988)).
21

22 The C8bp/HRF/MIP protein is attached to the cell
23 surface by means of a glycolipid anchor, as are CD59
24 and DAF: these proteins are known to be functionally
25 absent in paroxysmal nocturnal haemoglobinuria.
26

27 An 18-20 kDa protein is also implicated at the MAC
28 level. The following are believed to be identical (but
29 may not be):
30

31 P-18 (Sugita et al (J. Biochem 104 633 (1988)));

32

33 HRF-20 (Okada et al (Intl. Immunol 1 (1989)));

1
2 CO59 (Davies et al (J. Exp. Med. (Sept 1989)));
3 and
4

5 Membrane inhibitor of reactive lysis (MIRL)
6 (Hologuin et al J. Clin. Invest 84 7 (1989))).
7

8 The evidence for the putative identity of these
9 proteins is that the protein and/or cDNA sequences for
10 CD59 and HRF-20 are shown to be identical: probably
11 they are the same as P-18/MIRL also. It should be
12 noted that there is some homology of the CD59/HRF.20
13 sequence with that of murine LY-6 antigen, which is
14 involved in T-cell activation (Gronx et al (J. Immunol.
15 142 3013 (1989))).
16

17 SP-40.40 is also involved in MAC regulation (Kivszbaum
18 et al EMBO 8, 711 (1989)).
19

20 It is preferred that the HCRF interfere with complement
21 activation at the C3 stage. MCP and DAF both block the
22 positive feedback loop in the alternative pathway of C3
23 activation, and these constitute preferred HCRFs.
24

25 The HCRF is provided in association with the grafted
26 tissue. This means that the HCRF is administered in
27 such a way that the graft tissue is labelled as self,
28 but other foreign material, such as invading bacteria,
29 are not significantly so labelled. It may be possible
30 simply to administer parenterally, but locally to the
31 graft tissue, one or more appropriate HCRFs. However,
32 in practice this may not be preferred because of the
33 difficulty of causing adequate localisation of the HCRF

1 at the graft tissue and because of the further
2 difficulty that the HCRF may have to be repeatedly
3 administered to the recipient after the graft has taken
4 place; however, this could be overcome by the use of
5 specialist pharmaceutical delivery systems.

6
7 It will generally be much more convenient to provide
8 the HCRF in such a way that it is integrated with the
9 cell membrane on donor tissue. Although there may be
10 some benign infections of the transplanted tissue which
11 could cause suitable expression, by far the most
12 preferred route of achieving this end is for the donor
13 tissue to be transgenic in that it contains and
14 expresses nucleic acid coding for one or more HCRFs
15 active in the recipient species when grafted into the
16 recipient. Such transgenic tissue may continue to
17 express an HCRF indefinitely. The HCRF may be
18 genetically derived from the recipient species or less
19 preferably from a closely related species for which
20 concordant xenografts may be possible.

21
22 Although in principle the transgenic donor tissue may
23 come from a cell culture, it is preferable for the
24 donor tissue to come from a transgenic animal. The
25 transgenic animal should express (or be capable of
26 expressing) the HCRF in at least the tissue to be
27 transplanted, for preference. However, even this is
28 not essential, as it may be possible to bind the HCRF
29 to the cell membranes of the donor tissue by some
30 binding agent (such as a hybrid monoclonal antibody
31 (Milstein & Cuello Nature 305 537 (1983)) or receptor.

32

33

1 The recipient species will primarily be human, but not
2 exclusively. Other primates may be suitable
3 recipients, as may any other species where the
4 economics and ethics permit.

5
6 The donor species may be any suitable species which is
7 different from the recipient species and which, having
8 regard to the physiology of the recipient species is
9 able to provide appropriate tissue for transplantation.
10 For human recipients, it is envisaged that pig donors
11 will be suitable, but any other species may be
12 suitable.

13
14 According to a second aspect of the invention, there is
15 provided graftable animal cells or tissue of a donor
16 species, the cells or tissue being associated with one
17 or more homologous complement restriction factors
18 active in the intended recipient species to prevent the
19 complete activation of complement, the donor species
20 being a discordant species with respect to the
21 recipient species.

22
23 According to a third aspect of the invention there is
24 provided a transgenic animal having transplantable
25 tissue, which does not give rise to xenograft rejection
26 on transplantation into or exposure to the immuno
27 system of at least one discordant species. A
28 discordant species is one which would normally
29 hyperacutely reject a xenograft from the animal.

30
31 The invention therefore encompasses the use of animal
32 tissue derived from a donor species and one or more
33 homologous complement restriction factors active in a

1 recipient species, wherein the donor species is a
2 discordant species in relation to the recipient
3 species, in the preparation of tissue graftable into
4 the recipient species.

5
6 According to a fourth aspect of the invention, there is
7 provided a transgenic animal having cells capable of
8 expressing a homologous complement restriction factor
9 of another species. The homologous complement
10 restriction factor will generally be active in a
11 species which is discordant with respect to the species
12 of the transgenic animal. The cells may be of one
13 particular tissue, with preferences being as described
14 with reference to the first aspect of the invention, or
15 of more than one or all tissues, in which case the
16 animal may become a donor of more than one tissue.
17 Such a transgenic animal may be regarded as a
18 collection of non-transformed (in the sense of
19 non-proliferative) cells.

20
21 According to a fifth aspect of the invention, there is
22 provided a non-transformed animal cell capable of
23 expressing one or more homologous complement
24 restriction factors active in a species which is
25 discordant with respect to the animal cell.

26
27 According to a sixth aspect of the invention, there is
28 provided recombinant DNA comprising DNA coding for at
29 least one homologous complement restriction factor and
30 one or more sequences to enable the coding DNA to be
31 expressed by a non-transformed animal cell. The animal
32 cell may be a cell of a transgenic animal genetically
33 incorporating the construct. As an alternative, the

1 cell may be a cultured organ or other tissue such as an
2 Islet of Langerhans.

3

4 According to a seventh aspect of the invention, there
5 is provided a genetic construct suitable for
6 incorporation into the genetic material of an animal to
7 produce a transgenic animal, the construct comprising
8 DNA coding for at least one homologous complement
9 restriction factor and one or more sequences to enable
10 the coding DNA to be expressed in at least some cells
11 of a transgenic animal genetically incorporating the
12 construct. Such a genetic construct may be in the form
13 of a mini chromosome known as a YAC. As above, the
14 homologous complement restriction factor will generally
15 be active in a species which is discordant with respect
16 to the species of the transgenic animal.

17

18 According to a eighth aspect of the present invention,
19 there is provided a method of preparing a transgenic
20 animal, the method comprising incorporating into an
21 animal's genetic material DNA coding for at least one
22 homologous complement restriction factor and one or
23 more sequences to enable the coding DNA to be expressed
24 in at least some cells of the transgenic animal.

25

26 Methods of producing transgenic animals are in general
27 becoming more widespread, and the detailed steps to be
28 taken may be as now conventionally used in the art.
29 For example, WO-A-8800239 discloses the steps needed in
30 principle to construct a transgenic animal.

31

32 The actual method of incorporation of the construct
33 into the cells of the transgenic animal may be by

1 micro-injection, by sperm-mediated incorporation or any
2 other suitable method. The preliminary genetic
3 manipulation may be carried out in a prokaryote, as is
4 generally preferred.

5
6 DNA coding for HCRFs is either available in cDNA form
7 or may be deduced using conventional cloning
8 techniques. The DNA coding for decay accelerating
9 factor (DAF) is probably the best characterised and has
10 been described by Medof *et al* (*PNAS* 84 2007-2011
11 (1987)). A physical map of the RCA gene cluster is
12 given in Rey-Campos *et al* (1988) (*loc. cit.*). Variants
13 of DAF and their preparation by recombinant DNA
14 technology are disclosed in EP-A-0244267; such variants
15 may be used in the present invention.

16
17 Because of the better characterisation of the genetics
18 of DAF, and the known sequence of cDNA encoding DAF,
19 DAF constitutes a preferred homologous complement
20 restriction factor.

21
22 Other preferred features of the second to seventh
23 aspects of the invention are as for the first aspect,
24 mutatis mutandis.

25
26 The invention will now be illustrated by the following
27 examples. In the examples, reference is made to the
28 drawings in which:

29
30 FIGURES 1A to 1E show successive ECG traces for a
31 rabbit's heart grafted onto neonate pigs in
32 accordance with Example 1;
33

1 FIGURE 2 shows the result of a radioimmunoassay
2 indicating that the pigs used in Example 1 had no
3 significant amounts of antispecies antibody;

4
5 FIGURE 3 shows certain stages of protein
6 electrophoresis, as used in Example 4;

7
8 FIGURE 4 shows certain stages of two dimensional
9 crossed electrophoresis, as used in Example 4;

10
11 FIGURE 5 shows the "2D-Rockets" resulting from
12 Example 4;

13
14 FIGURE 6 shows the result of a chromium release
15 cell lysis assay in Example 5;

16
17 FIGURE 7 illustrates titres of lytic anti-hamster
18 antibodies from a rat recipient of a hamster heart
19 graft, pre-transplant (day 0) and days 5, 7 and 9
20 post-transplant, as described in Example 6;

21
22 FIGURE 8 shows graphically ODs of G200 fractions;
23 the histogram illustrates titres in each fraction
24 of lytic anti-hamster antibodies from a rat
25 recipient of a hamster heart, as described in
26 Example 6;

27
28 FIGURE 9 shows a Southern blot of DNA extracted
29 from T5, b10 and DB3 cell lines, as described in
30 Example 7;

31
32 FIGURE 10 shows ⁵¹Cr release figures, indicative
33 of T5 human cell line being lysed by rabbit

1 complement but not human complement in the
2 presence of pig anti-human antibodies, as
3 described in Example 7;

4

5 FIGURE 11 shows release figures, indicative of a
6 failure of human antibodies to lyse T5 human cell
7 line either with human or rabbit complement, as
8 described in Example 7;

9

10 FIGURE 12 shows ^{51}Cr release figures, which
11 demonstrate that human antibodies can lyse a
12 mouse-mouse hybridoma (DB3) in the presence of
13 both rabbit complement or human complement, as
14 described in Example 7;

15

16 FIGURE 13 shows ^{51}Cr release, illustrating that
17 the human-mouse hybrid cell line B10 is lysed by
18 human antibodies in the presence of rabbit
19 complement but not lysed by human antibodies in
20 the presence of human complement, as described in
21 Example 7;

22

23 FIGURE 14 shows uptake of ^3H adenine (in counts
24 per minute) by CHO cells, showing that these cells
25 are killed by immune rat serum in the presence of
26 human complement or rabbit complement, as
27 described in Example 8;

28

29 FIGURE 15 shows uptake of ^3H adenine in counts per
30 minute by CHO cells transfected with human MCP,
31 showing that these cells are killed by immune rat
32 serum in the presence of rabbit complement but are
33 not killed by this immune rat serum in the

1 presence of human complement, as described in
2 Example 8;

3
4 FIGURE 16 shows "2D rockets" showing that, in the
5 circumstances described in relation to Figure 15,
6 the C3 component of human complement is not
7 cleaved to form C3b, as described in Example 8;

8
9 FIGURE 17 shows ^{51}Cr release figures, indicative
10 of 3T3 mouse fibroblasts being lysed by naturally
11 occurring antibodies in the presence of human
12 complement and the protective effect of the
13 expression of human MCP by the mouse cells; and

14
15 FIGURE 18 shows a slot blot analysis of DNA of
16 second generation transgenic mice using labelled
17 MCP cDNA (upper) or labelled DAF cDNA as a probe.

18
19 EXAMPLE 1

20
21 Xenograft Rejection Takes Place in the Absence of any
22 Antispecies Antibodies

23
24 In general, animals cannot survive without circulating
25 immunoglobulins. These are produced by lymphocytes in
26 response to antigenic stimuli. In early neonatal life,
27 however, passively transferred maternal immunoglobulin
28 acts as a temporary substitute for this self-produced
29 antibody. This passively transferred immunoglobulin
30 confers protection on the young while early immune
31 experience is acquired. In mammals this passive
32 transfer of maternal immunoglobulin usually occurs both
33 transplacentally and via colostrum. In a few species,

1 however, the structure of the placenta is such that no
2 maternal antibody can be transferred by this route.
3 The pig is one such species. All maternal antibody is
4 obtained from colostrum. Thus, new born pre-suckled
5 pigs are in principle immunoglobulin-free.

6
7 Large white pigs were taken at birth and placed in a
8 wooden cage warmed by hot-water bottles without being
9 allowed to suckle. Two pigs from each farrowing were
10 taken for each experiment. These animals weighed
11 approximately 1kg at the time of birth.

12
13 Baby New Zealand white rabbits weighing approximately
14 300gms were used as donors. These donors were
15 anaesthetised with hypnol and diazepam, the chest was
16 opened and a vena cava cannulated by means of a 19
17 gauge needle. Cold (+4°C) cardioplegia (Thomas No. 2)
18 was infused until the heart stopped beating and had
19 become perfused with cardioplegia. Cooling was also
20 applied externally with cold cardioplegia directly from
21 a syringe. The rabbits heart was then removed using
22 standard surgical techniques and stored in cardioplegia
23 solution at +4°C until required. It has been found
24 necessary to take these precautions because the rabbit
25 heart proved to be highly susceptible to ischaemic
26 damage.

27
28 Recipient pigs were anaesthetised initially by
29 Halothane/O2 inhalation. An intravenous butterfly (23
30 gauge) needle was then inserted into a mammary vein,
31 anaesthesia maintained by intravenous ketamine. The
32 pig was simultaneously kept hydrated with intravenous
33

1 saline. Serum and EDTA blood samples were drawn
2 pre-transplant.

3
4 The rabbit heart was grafted into the neck of the pigs
5 after the method of Heron (Acta Pathol. Microbiol.
6 Scand. 79 366-372 (1971)). The aorta was anastomised
7 end to side (6-0 prolene) to the carotid artery and the
8 pulmonary artery anastomised to the jugular vein. All
9 other cardiac vessels were ligated. Hearts began
10 beating within a few minutes of removal of clamps.
11 Heart rate was monitored throughout by a diascope/ECG
12 monitor. The pig neck was not closed during the
13 experiments, hearts were kept moist by covering with
14 cling film.

15
16 The ECG results are shown in Figures 1A to 1E. The
17 trace shown in Figure 1A shows a normal heart beat
18 immediately after transplantation. Failure begins some
19 twenty minutes later (Figure 1B) and within an hour
20 (Figure 1D) there is no detectable heart beat,
21 evidencing hyperacute rejection.

22
23 This example therefore demonstrates that hyperacute
24 rejection of discordant xenograft takes place even in
25 the absence of antibodies.

26

27

28

29

30

31

32

3

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1 EXAMPLE 2

2

3 The Neonatal Pigs used in Example 1 have no Antispecies
4 Antibody

5

6 Rabbit anti-pig IgG was radioiodinated by the method of
7 Greenwood et al, Biochemical Journal 89 114-123 (1963)
8 modified by Davies and Howard (not published).

9

10 The following are added into a polystyrene tube (LP2
11 6 cm x 1 cm) in rapid succession:

12

13 25-50 μ l protein (at 1mg/ml conc)

14 3-4 μ l Na¹²⁵I (100 mCi/ml)

15 10 μ l chloramine-T (*4mg/5ml; 0.5M)

16 sodium phosphate buffer (pH 7.5)

17 * must be freshly prepared before use

18

19 These components were allowed to mix for 30 seconds
20 with continuous agitation. Then the following were
21 quickly added:

22

23 50 μ l DL-tyrosine (sat. sol. in 0.5ml sodium phosphate
24 buffer pH 7.5).

25

26 300 μ l 2% BSA/PBS/azide

27

28 The labelled protein is then separated from the
29 unreacted iodine, by the use of a small column
30 8cm x 1.0cm of Sephadex G-25 medium grade made up in
31 PBS/azide. The iodination reaction mixture is
32 quantitatively transferred to the prepared G25 column
33 and eluted with PBS/azide. Six drop fractions are

1 collected into polystyrene tubes (LP2). The column is
2 eluted until both the protein and the (^{125}I) iodide
3 peaks have been eluted and the radioactivity in all of
4 the fractions is measured.

5

6 The radioactivity incorporated into the protein can be
7 calculated thus:

8

9 radioactive counts in protein = original total
10 counts - counts in iodide peaks

11

12 The radiolabelled IgG (referred to now as "isotope") is
13 then used in an assay for (pig) antibodies in the
14 neonatal pig, as follows:

15

16 Materials

17

18 PBS + 0.01% Azide - Oxoid

19 PBS/BSA 1% - BSA-Sigma

20 Isotope - rabbit anti-pig IgG whole molecule with
21 $12-18 \times 10^3$ counts/min.

22 Heat inactivated sera (56°C 30 mins)

23 Anticoagulated blood samples.

24

25 Method

26

27 1. A 1% suspension of rabbit red blood cells in PBS
28 was prepared and 100 μl amounts were added to
29 tubes. Cells are spun to a button discarding
30 supernatant.

31

32 2. Serial dilutions of inactivated sera were prepared
33 in PBS/BSA from adult pig (positive control),

1 neonatal pig (test sample) or rabbit (negative
2 control). 0.025ml amounts were added to red cell
3 buttons in duplicate. Tubes were incubated at 4°C
4 for 4 hours.

5

6 3. After incubation tubes were washed three times in
7 PBS/BSA 0.05ml of Isotope was then added to each
8 tube and incubated overnight at 4°C.

9

10 4. Tubes were rewashed three times and 1 min counts
11 were performed on gamma counter.

12

13 5. Results are plotted as number of counts/min
14 against titre.

15

16 The results are shown in Figure 2. Rabbit serum was
17 used as a negative control and adult (ie suckled) pig
18 serum as a positive control. It can be seen that the
19 level of pig antibody in the pre-suckled pig 2 is
20 comparable to that of the negative control.

21

22 EXAMPLE 3

23

24 Demonstration of Relevance of Complement C3 to 25 Xenograft Destruction

26

27 Complement deficient guinea pigs derived from the
28 strain described by Burger *et al* (*Eur. J. Immunol* 16
29 7-11 (1986)) were grafted with hearts using essentially
30 the same technique as that described for the
31 rabbit-to-pig xenografts in Example 1. Rats were
32 anaesthetised with ether inhalation and hearts cooled
33 with cardioplegia and excised as previously described.

1 Guinea pig donors were anaesthetised with intravenous
2 valium and intramuscular hypnol. Hearts were implanted
3 into the neck as previously described. For control
4 guinea pigs, i.e. those with normal complement levels,
5 graft rejection normally took place within a few
6 minutes, thus making it unnecessary to close the neck.
7 In experimental animals the neck was closed and hearts
8 monitored by twice daily palpation. Normal ECGs were
9 observed for several hours post surgery, indicating no
10 hyperacute rejection.

11

12 EXAMPLE 4

13

14 A. Pig Lymphocytes and Kidney Cells Activate Human
15 Complement by the Alternative Pathway

16

17 Following the technique of Grabar and Williams
18 (Biochim. Biophys. Acta 10 193 (1953)), agarose gels 1
19 were poured onto 8 x 8cm glass plates (Figure 3). 10ml
20 of gel mixture was required, and this consisted of 5ml
21 2% agarose and 5ml veronal buffer (VB). (VB is 75mM Na
22 barbitone, 10mM EDTA, 10mM NaN₃, pH 8.5.) The agarose
23 and VB were mixed together at 60°C just before use.
24 Gels were poured and cooled on a level platform. When
25 set, the gel consisted of 1% agarose and had a depth of
26 about 1.5mm.

27

28 Wells 3 with a diameter of 3mm were cut about 1cm from
29 one end of the gel. Each well could contain about 8µl
30 of the sample to be run. The sample had no special
31 preparation apart from the addition of enough
32 bromophenol blue to colour it. After application of
33 the sample the gel was carefully placed onto the

1 platform of the electrophoresis tank. Cotton wicks
2 soaked in VB (the running buffer) were then gently
3 pressed along the edge of the gel nearest the wells,
4 and another wick was pressed onto the opposite edge of
5 the agarose. (It is important to ensure that the ends
6 of the wicks dip into the buffer reservoirs.) A
7 current of 25-30 mA was then passed through the gel
8 until the albumin (visualised with bound bromophenol)
9 reached the positive (anode) wick. The process takes
10 about two and a half hours to three hours. If two or
11 more gels are to be run simultaneously and in parallel
12 then the current applied must be increased accordingly
13 so that two gels required 50 mA and three require 75
14 mA, and so forth.

15
16 When electrophoresis was complete, as indicated by the
17 travel of an albumen marker 9 visualised with
18 bromophenol blue, the gel was removed from the
19 electrophoresis tank.

20
21 B. 2-Dimensional Crossed Immunelectrophoresis (2-D
22 Rockets)

23
24 Strips 11 (Figure 4) containing the electrophoresed
25 proteins from (A), were cut and laid at one end of a
26 new glass plate 13. A 1:1 mixture 15 of 2% agarose:VB
27 containing about 1% antiserum to the protein to be
28 visualised was then poured onto the plate and allowed
29 to set. The antiserum was added to the agarose/VBS
30 mixture when this had cooled to a temperature of about
31 50°C.

32
33

1 The rocket plate was then electrophoresed as described
2 above, with the end of the gel containing the 1st
3 dimension strips connected via a cotton wick to a
4 negative electrode (cathode) 17 and the opposite end
5 connected to an anode 19. The gels were
6 electrophoresed overnight at a current dependent on the
7 size of the gels; 10mA is needed for each 8cm length of
8 gel so that a gel of 16cm length requires 20 mA of
9 current, and so forth.

10

11 The proteins are separated by the electrophoresis in
12 the first dimension and quantified and visualised by
13 electrophoresis in the second dimension, staining for
14 the purpose of visualisation will now be described.

15

16 C. Squashing and Staining Gels

17

18 This procedure is the same for either conventional
19 immunoelectrophoresis or rockets. The gel to be
20 stained was covered with 1 layer of fibre-free POSTLIP
21 (Trade Mark) paper (Adlard Evans & Co), pre-moistened
22 with water. This was then covered with 6 layers of
23 absorbent paper towelling. The assembly was squashed
24 for 1 hour, after which all the paper was removed and
25 the process repeated.

26

27 After the second squash the gel was dried under a
28 current of warm air and then soaked in PBS for at least
29 1 hour to remove non-precipitated protein. The gel was
30 then dried again, and stained for 10 minutes in a
31 solution of 0.5% w/v coomasie brilliant blue G250, 45%
32 H₂O, 45% methanol, 10% acetic acid.

33

1 The gel was de-stained by continuous washing in 20%
2 methanol, 6% acetic acid until the background was
3 clear. It was then finally dried under warm air.

4
5 Figure 5 is a reproduction of the dry gel. Rocket 1 is
6 a negative control containing 50 μ l normal human serum
7 (HHS) plus 25 μ l VBS including 10mM EGTA. EGTA is a
8 chelator which removes calcium; calcium is essential
9 for classical pathway complement activation, and so the
10 presence of EGTA ensures that complement can only be
11 activated by the alternative pathway. The left-hand
12 (larger) peak is C3, and the right-hand (smaller) peak
13 is C3bi, a breakdown product of activated C3. In the
14 control, therefore, the small amount of C3bi indicates
15 only a minor amount of complement activation.

16
17 In Rocket 2, 75% pig erythrocytes (v/v) were added to
18 the buffer cocktail. There is a slight, but probably
19 not significant, increase in the C3bi level, thereby
20 indicating that pig erythrocytes only marginally, if at
21 all, activate human complement by the alternative
22 pathway. The reason for this poor response is not
23 clear.

24
25 In Rockets 3 and 4, 75% pig lymphocytes (v/v) or 75%
26 pig kidney cells (v/v), respectively, were added to the
27 buffer cocktail. In each case there was an appreciable
28 rise in the C3bi level, indicating activation of human
29 complement by the pig lymphocytes.

1 EXAMPLE 5

2

3 Pig Lymphocytes are not Lysed by Human Antibodies in
4 the Presence of Pig Complement, but are Lysed in the
5 Presence of Rabbit or Human Complement

6

7 A chromium release assay was used to monitor lysis of
8 cells mediated by human serum in the presence of either
9 pig complement, baby rabbit complement or human
10 complement.

11

12 Materials

13

14 Lymphocyte separation medium - Flowlabs
15 RPMI 1640 + 10% inact. FCS
16 PBS (without azide) - Oxoid
17 V wellled plates - Sterilin
18 Baby rabbit comp lymph - Sera - lab - or human or pig
19 complement (dilutes 1+7 in RPM1)
20 Heat inactivate sera (56°C 30 mins)

21

22 Method

23

24 1. Defibrinated whole pig blood, diluted 1:1 in PBS
25 was layered onto an equal volume of Ficoll Hypaque
26 lymphocyte separation medium. The tubes were spun
27 at 1200g for 30 mins at 20°C.

28

29 2. The resulting pig lymphocytes at the interface
30 were removed and washed once in PBS. The button
31 was resuspended in RPMI 1640 and the cell count is
32 adjusted to 2×10^7 /ml.

33

- 1 3. 200 μCi of ^{51}Cr were added to a 2×10^7 pellet of
2 cells and incubated at room temperature for 1.5
3 hours.
- 4
- 5 4. Labelled cells were washed twice at 900g for 5 min
6 uses and adjusted to give a final cell count of 1
7 $\times 10^6/\text{ml}$ in RPM1.
- 8
- 9 5. 0.05ml amounts of inactivated sera under test as
10 serial dilutions in duplicate, together with
11 controls, were plated out. Diluted complement was
12 added to relevant wells in 0.05ml amounts followed
13 by 0.05ml of labelled cells. Plates are incubated
14 for 1 hour at 30°C in a CO_2 oven.
- 15
- 16 6. After incubation, the plates were spun for 15 mins
17 at 900g 20°C to sediment the cells. $100\mu\text{l}$ of
18 supernatant is removed into labelled tubes and 1
19 minute counts are performed on gamma counter.
- 20
- 21 7. Results are plotted as a % of the count of the
22 original labelled cells against titre.
- 23

24 Controls

- 25
- 26 Full release control (FRC) - $50\mu\text{ls}$ cells + $100\mu\text{ls}$
27 H_2O + 0.1% + Tween
- 28 Negative control - $50\mu\text{ls}$ cells + $100\mu\text{ls}$ RPM1
- 29 Complement control (CC) - $50\mu\text{ls}$ cells + $50\mu\text{ls}$ dil'd.
30 comp. + $50\mu\text{ls}$ RPM1
- 31
- 32
- 33

1 Results

2

3 The results are shown in Figure 6. It can be seen that
4 pig lymphocytes are lysed by human serum only in the
5 presence of non-pig (ie rabbit or human) complement,
6 but not in the presence of pig complement. The
7 inference is that one or more homologous complement
8 restriction factors present on pig cells successfully
9 down-regulate the action of pig complement but not the
10 action of human or rabbit complement.

11

12 EXAMPLE 6

13

14 The purpose of this example is to demonstrate that
15 antibody can cause hyperacute rejection. The concept
16 upon which this application is based arose as a result
17 of the observation that hyperacute rejection may take
18 place in the absence of anti-graft antibodies but
19 requires functional complement. Because this is a
20 novel observation there are no experiments in the
21 literature which formally demonstrate that antibody can
22 cause xenograft rejection. Since in the presence of
23 naturally occurring antibody it is difficult to
24 determine whether these antibodies are playing a role
25 or not such an experiment is not easy to perform. In
26 this example the role of antibody has been demonstrated
27 by turning a concordant xenograft into a discordant
28 xenograft by infusion of antibody of appropriate
29 specificity. Recipients used in this study were male
30 rats of the PVG strain (RT1C) (Banting & Kingdom,
31 Bicester, Oxon., UK) between 3 and 6 months old
32 weighing 250-300 g. Heart donors were Syrian hamsters
33 also obtained from Banting & Kingdom and weighing

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1 between 100 and 150 g. Heart grafting was performed
2 according to the method of Heron (loc. cit. in Example
3 1). Hamster hearts were grafted into the neck of the
4 rats joining the aorta to the carotid artery and the
5 pulmonary artery to the jugular vein by means of a cuff
6 technique. All other vessels were ligated. Hearts
7 started beating minutes after the release of vascular
8 clamps and were monitored by external palpation. All
9 operations were carried out on animals anaesthetised by
10 inhalation of halothane and oxygen.

11
12 Anti-hamster lytic antibody levels were measured as
13 follows: 50 μ l of 1% hamster erythrocyte solution were
14 added to 50 μ l of test serum which had been diluted
15 serially. 50 μ l of a 1 in 7 dilution of baby rabbit
16 complement (Sera Lab, Crawley Down, Sussex) were added
17 and incubated for 1 hour at 37°C. 750 μ l of complement
18 fixation diluent were added and centrifuged (Beckman
19 MICROFUGE, 13000 rpm for 4 minutes) after which the
20 OD₄₁₅ was measured in the supernatant. (The word
21 MICROFUGE is a trade mark.) Positive and negative
22 controls were CFD and distilled water added to a 1%
23 solution of cells respectively. The results of the
24 OD₄₁₅ readings were plotted against the serum titration
25 on the x-axis. As can be seen from Figure 7, grafting
26 a hamster heart into a rat results in the rat producing
27 very high titres of lytic anti-hamster antibodies.
28 Sera from some of these rats were separated into their
29 component protein fractions by column chromatography on
30 SEPHADEX G200 (Pharmacia GB Ltd, London) using standard
31 column chromatography techniques ("The use of SEPHADEX
32 in the separation, purification and characterisation of
33 biological materials", Curling in Exp. in Physiol. and

1 Biochem. 3 (1970) 417-484 (G.A. Kerkut, Ed.) Academic
2 Press, London and New York, 1970). (The word SEPHADEX
3 is a trade mark.) Each of the 7ml fractions collected
4 from the column were assayed for lytic anti-hamster
5 activity as described above. Figure 8 demonstrates
6 that despite the fact that these antibodies were
7 induced as a result of heart grafting the anti-species
8 activity resides almost exclusively in the IgM
9 fraction. After assaying for activity, fractions were
10 concentrated using CX10 ultrafilters (Pharmacia) to a
11 concentration of 0.5 mg/ml and stored at -70°C until
12 used.

13

14 To test for their ability to destroy a xenograft as
15 opposed to just lysing red cells, hamster hearts were
16 grafted into the necks of naive rats. As soon as the
17 hamster heart beat was established either 2ml of neat
18 serum or 0.5 mg of purified immunoglobulin containing
19 lytic anti-hamster antibodies were infused intra-
20 venously into the rat. Both the unseparated serum and
21 the 0.5 mg of IgM consistently caused the hamster heart
22 graft to be destroyed within 15 minutes. Results from
23 infusion of IgG were inconsistent with some
24 preparations causing the graft to fail while, in
25 others, the graft continued to beat. When albumin from
26 the G200 column was infused as a control heart grafts
27 always survived and were rejected in the normal time
28 for this model which is 3 days. This demonstrates that
29 the binding of this antibody to a graft can induce its
30 hyperacute destruction.

31

32

33

1 EXAMPLE 7

2

3 The data so far presented in this application have
4 demonstrated that the destruction of a xenograft can
5 involve complement activation either by the alternative
6 pathway or by antibody-mediated complement activation
7 (the classical pathway). Furthermore, complement
8 regulators on the surface of the xenograft target can
9 protect it from destruction by homologous but not
10 heterologous complement. The critical activation step
11 common to both complement activation pathways is the
12 cleavage of the complement component C3. This cleavage
13 is brought about by the C3 convertase, C4b2a (the
14 classical pathway C3 convertase) or the convertase
15 C3bBb (the alternative pathway C3 convertase). These
16 enzymes cleave C3 to C3b which, in turn, can engage the
17 alternative pathway to form more C3 convertases (the
18 feed-back loop). As a result the complement system is
19 rapidly able to amplify the deposition of C3b on a
20 "foreign" target. Much of the C3b however does not
21 successfully interact with the foreign target and
22 remains in the fluid phase and can thus
23 indiscriminately bind to the cells of the host. In
24 order to protect these cells from attack by the
25 indiscriminate binding of complement, control proteins
26 have evolved to inactivate complement components either
27 in the fluid phase or bound to self tissues. Those
28 glycoproteins which are involved in controlling C3 are
29 genetically all associated within one region of human
30 chromosome 1 called the RCA (regulators of complement
31 activation) locus. In this example we demonstrate that
32 mouse cells which have acquired through fusion
33 techniques the human chromosome 1 and express proteins

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1 of the RCA locus on their surface behave in an in vitro
2 assay of xenograft destruction as though they were
3 human cells and not mouse cells.

5 Cell Lines

7 T5 is an Epstein Barr virus-transformed tonsil B-cell
8 line produced by the technique of Bird et al. (Nature
9 289 300-301 (1981)). B10 is a human anti-tetanus
10 monoclonal antibody producing hybridoma which was
11 derived from the fusion of a human B lymphoblastoid
12 line (BLL) with the mouse myeloma cell line X63-AG8.653
13 (Kierney et al. (J. Immunol. 123 1548-1550 (1979))). T5
14 and B10 cell lines are obtainable from Ms C Carter and
15 Dr N C Hughes-Jones of the MRC MITI Group at Babraham,
16 Cambridge. DB3 is a mouse hybridoma cell line which
17 produces anti-progesterone monoclonal antibody (Wright
18 et al. Nature 295 415-417 (1982)). The following
19 oligonucleotide primers specific for human chromosome 1
20 were procured: (5'-CCACAGGTGTAACATTGTGT-3') [SEQ ID
21 NO: 1] and (5'-GAGATAGTGTGATCTGAGGC-3') [SEQ ID NO: 2];
22 these are, respectively, upstream and downstream
23 primers of human antithrombin 2 (AT3) gene known to be
24 on human chromosome 1 (Wu et al. Nucl. Acids Res. 17
25 6433 (1989)). The oligonucleotides can be synthesised
26 by techniques well known to those skilled in the art.

28 High molecular weight genomic DNA was prepared using
29 the method of Herrmann and Frischauf (Methods Enzymol.
30 152 180-183 (1987)). In brief, 100×10^6 cells from each
31 cultured cell line were lysed by 5ml of TNE (100mM Tris
32 pH 7.5, 100mM NaCl, 10mM EDTA 1% Sarkosyl) and treated
33 with fresh proteinase K (100 micrograms per ml). The

1 preparation was extracted with phenol (water saturated
2 and equilibrated against 0.1M Tris, pH 8) phenol
3 chloroform (1:1, V/V) and then chloroform isoamyl
4 alcohol (24:1 V/V). DNA was obtained by ethanol
5 precipitation and dialysed against TE (10mM Tris pH8.0,
6 1mM EDTA) made to 100mM in NaCl and TE alone at 4°C.
7 Isolated DNA was analysed on 0.5% agarose gel and the
8 concentration determined by optical density at 260 nm.
9 The polymerase chain reaction (PCR) for each cell line
10 was performed as described by Saiki et al. (Science 239
11 487-491 (1988)). In a volume of 100 µl containing
12 500 ng of genomic DNA 1.2 ng of each primer and 2.5
13 units of Taq DNA polymerase (Thermos acquaticus type 3)
14 (Cambio Ltd, Cambridge, UK) using the buffer supplied
15 with the enzyme. The nucleotides (dNTPs) (Boehringer
16 Mannheim Diagnostics and Biochemicals Ltd, Lewis, East
17 Sussex, UK) were at a concentration of 2mM each. DNA
18 was amplified for 30 cycles using a programmable thermal
19 controller (Genetic Research Instrumentation Ltd,
20 Dunmow, Essex, UK): denaturing 93°C 1 minute: annealing
21 55°C 1 minute: and extension 72°C 2 minutes. 10 µl of
22 the reaction product were analysed directly on a 2%
23 agarose gel run in Tris boric acid EDTA buffer. The
24 product size was determined by comparison with HincII
25 digested phage X-174-rf DNA (Pharmacia LKB
26 Biotechnology, Upsala, Sweden).
27
28 Cultured T5, B10 and DB3 cells were treated with
29 anti-DAF (decay accelerating factor) monoclonal
30 antibody (Kinoshita et al ((J. Exp. Med. 162 75-92
31 1985)) and fluorescein-conjugated second antibody.
32 Cells (1×10^6) were reacted with mouse anti-DAF
33 monoclonal antibody 1A10 (IgG2a 10 µg/ml in 100 µl of

1 10% FCS 0.1% azide). 1A10 (Kinoshita et al. (J.
2 Immunol. 136 3390-3395 (1986))) was obtained from Dr M
3 Davitz of New York University Medical Centre, New York
4 USA. Blank controls were buffer alone. After
5 incubation for 2 hours on ice the cells were washed 3
6 times, re-suspended and incubated in 100 μ l buffer
7 containing 1 in 100 FITC-conjugated goat F(ab')₂
8 anti-mouse IG (heavy and light chains affinity purified
9 and human IG absorbed) (Tago Immunochemicals Inc,
10 Burlingame, California, USA) for one hour on ice. Some
11 cells were incubated only with the second antibody as
12 staining controls. Since DB3 is a mouse IgG1-secreting
13 cell line, FITC-conjugated sheep anti-mouse IgG2A (1 in
14 40, The Binding Site Ltd, Birmingham, UK) or the goat
15 anti-mouse IG preabsorbed with equal volumes of DB3
16 cells were also used in order to eliminate anti-IgG1
17 reactivity occurring when staining DB3 cells. All the
18 cells were extensively washed and resuspended in 200 μ l
19 of buffer. DAF positive cells were detected using a
20 Beckton Dickinson FACS-STAR apparatus for
21 fluorescence-activated cell sorting (FACS) analysis.
22 (The expression FACS-STAR is a trade mark.)
23

24 The PCR method was used to determine the presence of
25 human chromosome 1 in three different cultured cell
26 lines, T5 (human), B10 (human-mouse) and DB3
27 (mouse-mouse). Figure 9 shows that after amplification
28 both T5 and B10 had a band size of 495 base pairs
29 whereas DB3 (ie the mouse-mouse hybrid) had no band at
30 all. It has been reported that PCR products using AT3
31 primers consisted of 2 alleles, sized 572 base pairs
32 (allele 1) and 496 base pairs (allele 2) (Wu et al.,
33 loc. cit.). The bands found in T5 and B10 genomic DNAs

1 correspond to allele 2. This demonstrates that the
2 human mouse hybrid cell line B10 contained human
3 chromosome 1.

4
5 FACS analysis for the presence of DAF showed that the
6 majority of the human T5 cells (85.7%) stained positive
7 with anti-DAF monoclonal antibody. A similar level
8 (83.1%) of positive cells was found in the mouse/human
9 hybrid B10 cells. The mouse-mouse hybrid DB3 cells
10 showed identical staining patterns for both anti-DAF
11 treated and untreated preparations. However, this
12 anti-mouse IgG1 reactivity was removed if (1)
13 FITC-conjugated sheep anti-mouse IgG2a was used or (2)
14 the above goat anti-mouse IgG was preabsorbed with DB3
15 cells. The results indicate that human-mouse hybrid
16 cell line B10 express human DAF on the cell membrane
17 surface as detected by specific anti-DAF monoclonal
18 antibodies. The level of expression is the same as for
19 the human cell line T5. A mouse-mouse hybridoma cell
20 line does not express human DAF.

21
22 Chromium release cytotoxic cell killing studies were
23 performed on these cell lines as is described in
24 Example 5 above. Figure 10 shows that, when pig
25 anti-human antibodies are incubated with the T5 human
26 cell line the addition of rabbit complement caused
27 lysis whereas no lysis occurs when human complement is
28 added because, of course, the T5 cell line will possess
29 human HCRFs. This is confirmation of the results of
30 Example 5. When human antibodies are used on the human
31 cell line no lysis occurs either with human complement
32 or with rabbit complement, showing there are no
33 auto-antibodies. The chromium release technique does

1 not allow for incubations to be continued long enough
2 to detect any significant levels of alternative pathway
3 activation of the rabbit complement by the human cells
4 (Figure 11). However, when human antibodies are
5 incubated with the DB3 mouse-mouse hybridoma cell line
6 (Figure 12), cell killing is achieved by both rabbit
7 complement and human complement demonstrating that
8 indeed human complement can function in such an assay.
9 When the B10 human-mouse hybrid, possessing human
10 chromosome 1 and known to be expressing at least DAF,
11 was used then rabbit complement caused lysis of the
12 cell line whereas human complement fails to cause lysis
13 of the cell line (Figure 13). The explanation for this
14 is that the human HCRFs being expressed by virtue of
15 possession of chromosome 1 on the mouse-human hybrid
16 have inhibited the activity of the human complement.
17

18 EXAMPLE 8

19
20 The preceding example demonstrates that possession of
21 chromosome 1 can prevent xenograft cell destruction.
22 While this is strong circumstantial evidence that it is
23 the CRA locus which is protecting the mouse cell from
24 xenograft destruction this example provides formal
25 proof. In this example, the effect of transfecting
26 non-human cell lines with human MCP and exposing them
27 to human or rabbit complement is demonstrated.
28

29 cDNAs were produced for MCP as described in detail by
30 Lublin et al. (J. Exp. Med. 168 181-194 (1988)).
31 Construction of transfected cell lines was performed
32 using the expression plasmid SFFV.neo using the
33 technique described by Fuhlbrigge et al. (Proc. Natl.

1 Acad. Sci. 85 5649-5653 (1988)). This contains the
2 Friend spleen focus forming virus 5' long terminal
3 repeat (SFFV.LTR) (Clark and Mak (Nucl. Acids Res. 10
4 3315-3330 (1982)) and (Proc. Natl. Acad. Sci. 80
5 5037-5041 (1983))). Cell lines were obtained from the
6 American Type Culture Collection 12301 Parklawn Drive,
7 Rockville, Maryland, USA. Cell lines used were CHO-K1
8 (ATCC CCL 61) and NIH/3T3 (ATCC CRL 1658). Expression
9 of the gene was confirmed using a monoclonal antibody
10 to MCP (Andrews et al. Ann. Hum. Genet. 49 31-39
11 (1985)) and FACS analysis as already described. In
12 some cases cell lines were selected for high level
13 expression of MCP by cell sorting on the FACS using
14 standard techniques.

15

16 This example illustrates the effect of transfecting CHO
17 cells with MCP. Because these cell lines grow as
18 monolayers, cell killing was assessed by the terminal
19 adenine uptake assay as described by de Bono et al.
20 (Immunology 32 221-226 (1977)). In brief, this assay
21 involved incubation of cell cultures in flat-bottomed
22 sterile 96 well plates with complement and antibody. At
23 the end of the experimental incubation period, cell
24 viability is assessed by the ability of the culture to
25 take up radioactive adenine. Viable cells will take up
26 the adenine, dead cells will not; thus viable cells
27 have high counts, dead cells have low counts.

28

29 In common with many transformed cells, CHO is
30 insensitive to naturally occurring antibodies and the
31 action of the alternative pathway of complement.
32 However, these cells are sensitive to those antibodies
33 which as has been demonstrated cause hamster heart

1 xenograft destruction as described in Example 6. Since
2 CHO cells are derived from hamster, these antibodies
3 killed the CHO cells with both human and rabbit
4 complement (Figure 14). When CHO cells are transfected
5 with human MCP, the cells can only be lysed in the
6 presence of rabbit complement. Human complement has
7 been inhibited by the presence of the human MCP on the
8 surface of the hamster cell line (Figure 15). Evidence
9 that the failure of the cells to be killed is indeed
10 due to a failure of C3 convertase is provided by
11 analysis of the breakdown of the human C3 after
12 incubation of the CHO cells by rocket
13 immuno-electrophoresis as described in Example 4 above.
14 As can be seen, no breakdown occurs above complement
15 only control levels (Figure 16).

16
17 These data confirm that genetically engineering
18 complement down-regulatory proteins on the surface of
19 non-human cells will protect those cells from the
20 mechanisms of hyperacute xenograft destruction which
21 have, as a common feature, a requirement for the
22 cleavage of the C3 component of complement.

23

24 EXAMPLE 9

25

26 Following the procedure of Example 8, 3T3 mouse
27 fibroblast cells were transfected with cDNA coding for
28 MCP (MCP clone K5.23). ⁵¹Cr was added to the cells as
29 described in Example 5. One volume of cells was then
30 incubated with one volume of human heat-inactivated
31 complement and one volume of human complement
32 pre-absorbed at 4°C with mouse spleen cells to remove
33 anti-mouse antibody from the human complement. The

1 mixture and serial dilutions with complement were
2 plated out. General conditions and features of the
3 chromium release assay are as described in Example 5.
4 The results for clone K5.23 are shown in Figure 17,
5 which also shows, as a control, the effect of the MCP
6 cDNA being introduced in the reverse orientation (in
7 which case it is not transcribed). Correctly
8 transcribed MCP cDNA confers protection on the cells
9 from killing, as evidenced by the relatively low level
10 of ^{51}Cr release, whereas non-transcribed cDNA does not
11 confer significant protection, as evidenced by the
12 relatively high level of ^{51}Cr release.

13

14 EXAMPLE 10

15

16 Similar results to those described in Example 8 above
17 can be obtained with L1/210 cells (a mouse leukaemic
18 cell line) transfected with with the cDNA for DAF.
19 cDNAs were produced for DAF as described in Lublin &
20 Atkinson (Ann. Rev. Immunol. 7 35-58 (1989)).

21

22 EXAMPLE 11

23

24 cDNA for MCP was prepared and ligated into SFFV.neo, as
25 in Example 8 above.

26

27 Using this DNA preparation transgenic mice were
28 produced as described in Manipulating the Mouse Embryo,
29 A Laboratory Manual by B. Hogan et al, Cold Spring
30 Harbour Laboratory (1986). Ten to fifteen (CBAXB10)f1
31 female mice, 3-4 weeks old, were induced to
32 superovulate by intraperitoneal injection of 5 units
33 serum gonadotrophin from pregnant mares (supplied

1 commercially as Folligon) followed 48 hours later by
2 intraperitoneal injection of 5 units chorionic
3 gonadotrophin from human pregnancy urine (supplied
4 commercially as Chorulon). The females were put to
5 mate, on the day of the Chorulon injection, with
6 (CBAXB10)F1 males and the next day females with vaginal
7 plugs were killed by cervical dislocation and
8 fertilized ova were isolated from their oviducts.

9
10 Three to four hundred ova, isolated in this way,
11 contained two pronuclei clearly visible under Nomarski
12 differential interference contrast optics at 400 x
13 magnification. One of the two pronuclei was injected
14 with approximately 2000 copies of the DNA preparation
15 containing the MCP cDNA transgene in concentrations
16 ranging from 0.5 to 2 ng/ μ l.

17
18 Ova that survived the microinjection were reimplanted
19 into the oviducts of (CBAXB10)F1 females that had mated
20 the previous night with vasectomized males and were
21 therefore pseudopregnant (ie, they had ovulated and
22 their hormonal state was that of pregnancy but their
23 own oocytes had not been fertilized). Approximately 30
24 microinjected ova were transferred to the oviducts of
25 each pseudopregnant female, under anaesthesia, either
26 on the same day of microinjection or the next day when
27 the ova were at the 2-cell stage. Normal gestation
28 ensued and seventeen mice were born from ten mothers.
29 Screening of the offspring was done by slot blot and/or
30 Southern blot (see Example 8), and also PCR, analysis
31 of DNA from tail skin cells, utilizing ^{32}P -labelled
32 probes and primers that recognize the transgene. One
33

1 of the offspring, a male, proved to be transgenic for
2 the MCP DNA sequence.

3

4 EXAMPLE 12

5

6 The procedure of Example 11 was repeated, except that
7 the cDNA for DAF, as described in Example 10, was used
8 in place of the cDNA for MCP. Twenty three offspring
9 were born from ten mothers. Three of them (two female,
10 one male), transgenic for DAF, were obtained, as shown
11 by Southern blotting.

12

13

14 EXAMPLE 13

15

16 The male mouse obtained in Example 11, containing a
17 human MCP cDNA transgene was allowed to grow to
18 maturity and mated with a (CBAXB10)F1 female. Eleven
19 offspring resulted. Tail cell DNA from each offspring
20 was screened by slot-blot analysis, using labelled
21 human MCP cDNA as a probe, to determine whether the
22 transgene had been inherited. The results are shown in
23 the upper part of Figure 18. It can be seen that
24 offspring 0, 1, 5, 7, 8 and 10 have inherited. (Four
25 controls were undertaken: human DNA (H); mouse DNA
26 (M); mouse DNA mixed with 10 pg human MCP labelled
27 cDNA; and mouse DNA mixed with 100 pg human MCP
28 labelled cDNA.)

29

30 EXAMPLE 14

31

32 The male mouse obtained in Example 12, containing a
33 human DAF cDNA transgene was allowed to grow to

1 maturity and mated with a (CBAXB10)F1 female. For each
2 of the resulting offspring, tail cell DNA was screened
3 by slot-blot analysis, using labelled human DAF cDNA as
4 a probe, to determine whether the transgene had been
5 inherited. The results are shown in the lower part of
6 Figure 18. It can be seen that offspring 13.3 (a
7 female) has inherited. (Four controls were undertaken:
8 human DNA (H); mouse DNA (M); mouse DNA mixed with 10
9 pg human DAF labelled cDNA; and mouse DNA mixed with
10 100 pg human DAF labelled cDNA.)

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1 SEQUENCE LISTING

2

3 SEQ ID NO: 1

4 SEQUENCE TYPE: Nucleotide

5 SEQUENCE LENGTH: 20

6

7 PROPERTIES: Upstream primer of human antithrombin 2
8 (AT3) gene

9

10 SEQUENCE:

11

12 CCACAGGTGT AACATTGTGT

20

13

14

15

16

17 SEQ ID NO: 2

18 SEQUENCE TYPE: Nucleotide

19 SEQUENCE LENGTH: 20

20

21 PROPERTIES: Downstream primer of human antithrombin 2
22 (AT3) gene

23

24 SEQUENCE:

25

26 GAGATAGTGT GATCTGAGGC

20

27

28

29

30

31

32

33

1 CLAIMS

2

3 1. A method of transplanting animal tissue into a
4 recipient, wherein the tissue is derived from a donor
5 of a different species from the recipient, the donor
6 species being a discordant species with respect to the
7 recipient, the method comprising grafting the tissue
8 into the recipient and providing in association with
9 the grafted tissue one or more homologous complement
10 restriction factors (HCRFs) active in the recipient
11 species to prevent the complete activation of
12 complement.

13

14 2. A method as claimed in claim 1, wherein the tissue
15 is an organ.

16

17 3. A method as claimed in claim 2, wherein the organ
18 is a heart, lung, liver, kidney, pancreas or thyroid.

19

20 4. A method as claimed in claim 1, wherein the tissue
21 comprises blood or haematopoietic cells, Islets of
22 Langerhans, brain cells or cells from endocrine
23 organs.

24

25 5. A method as claimed in any one of claims 1 to 4,
26 wherein the HCRF interferes with that part of the
27 complement activation cascade which is common to both
28 the classical and alternative pathways.

29

30 6. A method as claimed in any one of claims 1 to 5,
31 wherein the HCRF is a natural HCRF.

32

33

1 7. A method as claimed in claim 5, wherein the HCRF
2 regulates complement activation at C3.

3
4 8. A method as claimed in claim 7, wherein the HCRF
5 is or has the activity of:

6
7 Factor I (also previously known as C3b inactivator
8 or KAF);

9
10 Factor H;

11
12 C4 binding protein;

13
14 DAF (also known as CD55);

15
16 Membrane Cofactor Protein (MCP; also known as CD46
17 and first described as gp45-70 and further known
18 as gp66/56);

19
20 CR1 (also known as C3b/C4b receptor or CD35);
21 and/or

22
23 CR2 (also known as CD21, C3dg receptor, 3d/EBV
24 receptor and p140).

25
26 9. A method as claimed in any one of claims 1 to 8,
27 wherein the HCRF has the activity of a natural HCRF
28 whose gene is located in the RCA (regulator of
29 complement activation) locus, which maps to band q32 of
30 chromosome 1.

31
32 10. A method as claimed in claim 5, wherein the HCRF
33 regulates complement activation at C8 and/or C9.

1 11. A method as claimed in claim 10, wherein the HCRF
2 is or has the activity of:

3

4 C8bp (also known as HRF or MIP);

5

6 P-18 (also known as HRF-20, CD59 or MIRL); or

7

8 SP40.40.

9

10 12. A method as claimed in any one of claims 1 to 11,
11 wherein the HCRF is membrane bound.

12

13 13. A method as claimed in any one of claims 1 to 12,
14 wherein the HCRF is provided in such a way that it is
15 integrated with the cell membrane on donor tissue.

16

17 14. A method as claimed in claim 13, wherein the
18 donor tissue is transgenic in that it contains and
19 expresses nucleic acid coding for one or more HCRFs
20 active in the recipient species when grafted into the
21 recipient.

22

23 15. A method as claimed in any one of claims 1 to 14,
24 wherein the recipient species is human.

25

26 16. A method as claimed in any one of claims 1 to 15,
27 wherein the donor species is a pig.

28

29 17. Graftable animal cells or tissue of a donor
30 species, the cells or tissue being associated with one
31 or more homologous complement restriction factors
32 active in the intended recipient species to prevent the
33 complete activation of complement, the donor species

1 being a discordant species with respect to the
2 recipient species.

3

4 18. A transgenic animal having transplantable tissue,
5 which does not give rise to xenograft rejection on
6 transplantation into or exposure to the immuno system
7 of at least one discordant species.

8

9 19. The use of animal tissue derived from a donor
10 species and one or more homologous complement
11 restriction factors active in a recipient species,
12 wherein the donor species is a discordant species in
13 relation to the recipient species, in the preparation
14 of tissue graftable into the recipient species.

15

16 20. A transgenic animal having cells capable of
17 expressing a homologous complement restriction factor
18 of another species.

19

20 21. A non-transformed animal cell capable of
21 expressing one or more homologous complement
22 restriction factors active in a species which is
23 discordant with respect to the animal cell.

24

25 22. Recombinant DNA comprising DNA coding for at least
26 one homologous complement restriction factor and one or
27 more sequences to enable the coding DNA to be expressed
28 by a non-transformed animal cell.

29

30 23. DNA as claimed in claim 22, wherein the animal
31 cell is a cell of a transgenic animal genetically
32 incorporating the construct.

33

1 24. DNA as claimed in claim 22, wherein the cell is a
2 cultured organ or other tissue such as an Islet of
3 Langerhans.

4
5 25. A genetic construct suitable for incorporation
6 into the genetic material of an animal to produce a
7 transgenic animal, the construct comprising DNA coding
8 for at least one homologous complement restriction
9 factor and one or more sequences to enable the coding
10 DNA to be expressed in at least some cells of a
11 transgenic animal genetically incorporating the
12 construct.

13
14 26. A genetic construct as claimed in claim 25, which
15 is in the form of a YAC.

16
17 27. A method of preparing a transgenic animal, the
18 method comprising incorporating into an animal's
19 genetic material DNA coding for at least one homologous
20 complement restriction factor and one or more sequences
21 to enable the coding DNA to be expressed in at least
22 some cells of the transgenic animal.

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1/12

FIG. 1A

3.15

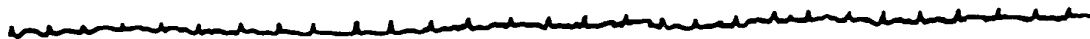


FIG. 1B

3.35

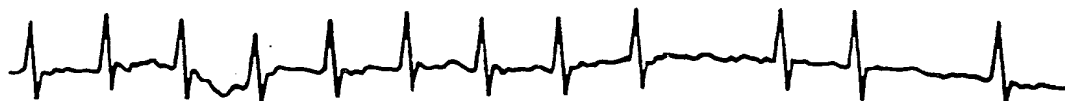


FIG. 1C

3.55



FIG. 1D

4.15

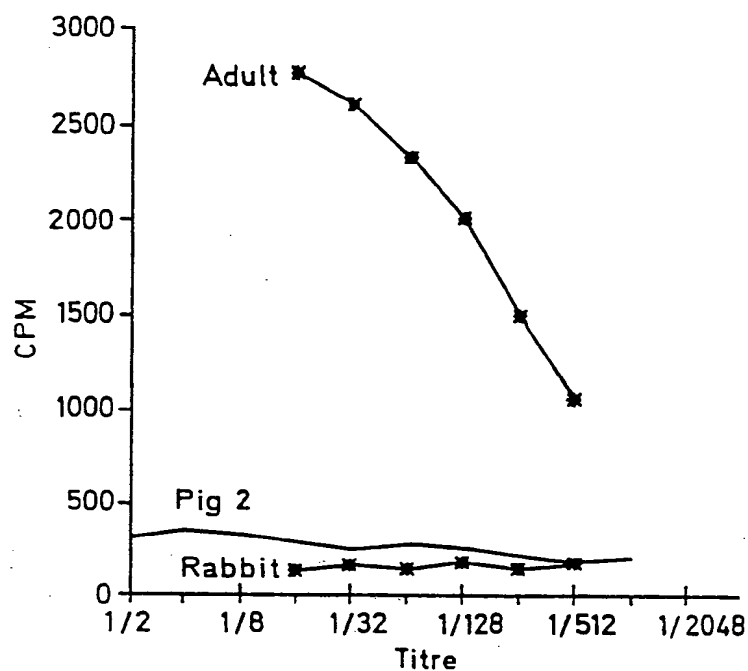
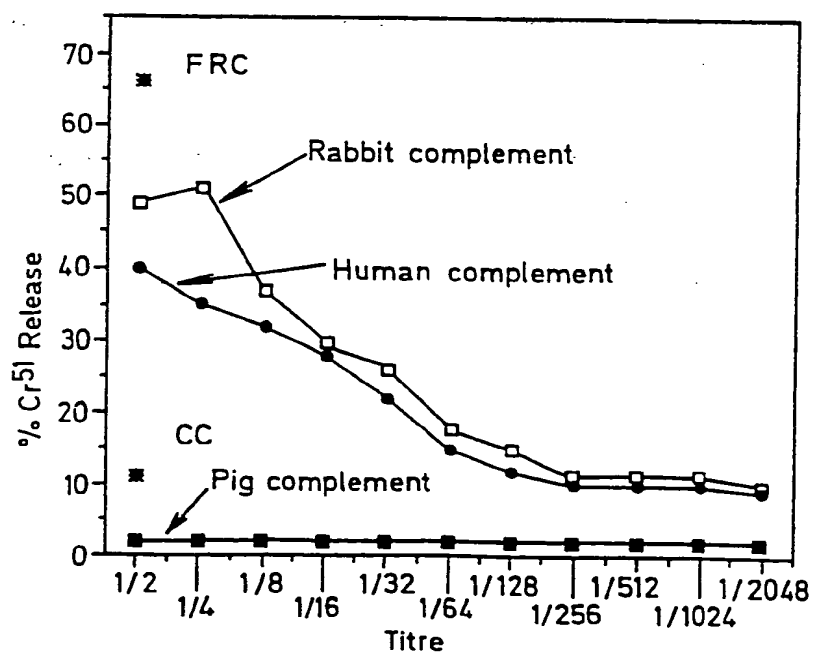


FIG. 1E

4.25



2/12

FIG. 2 Rabbit anti Pig antibody titres
Pre suckled pigs**FIG. 6**

3/12

FIG. 3

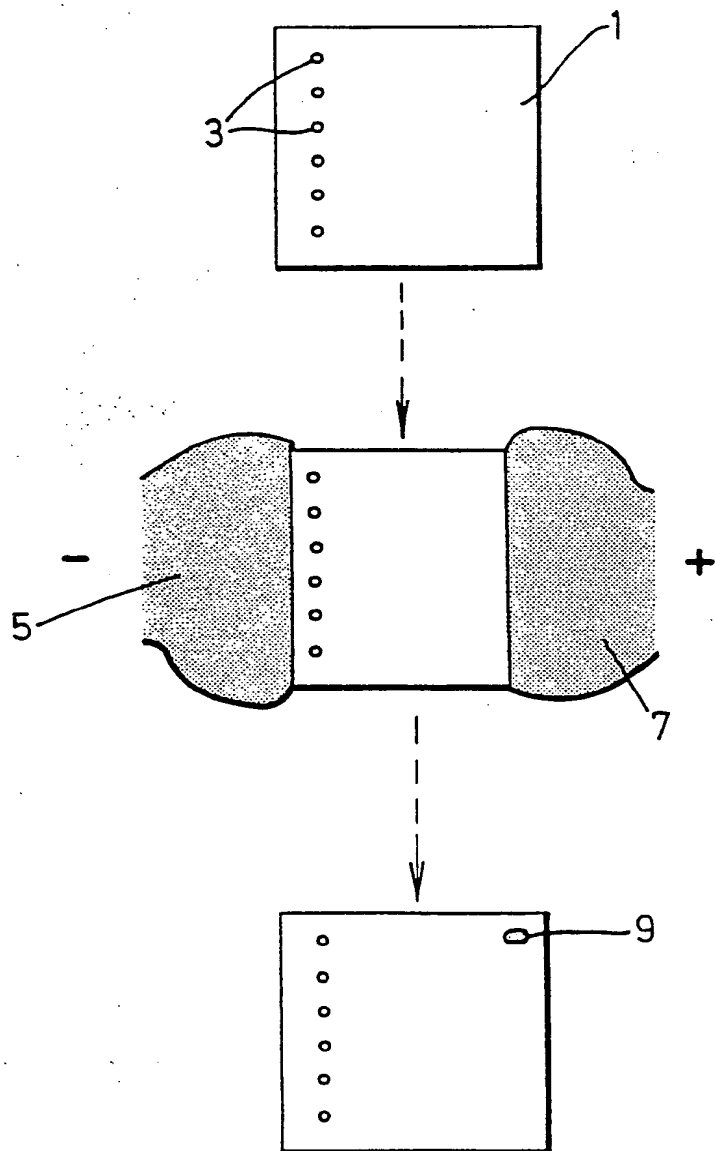
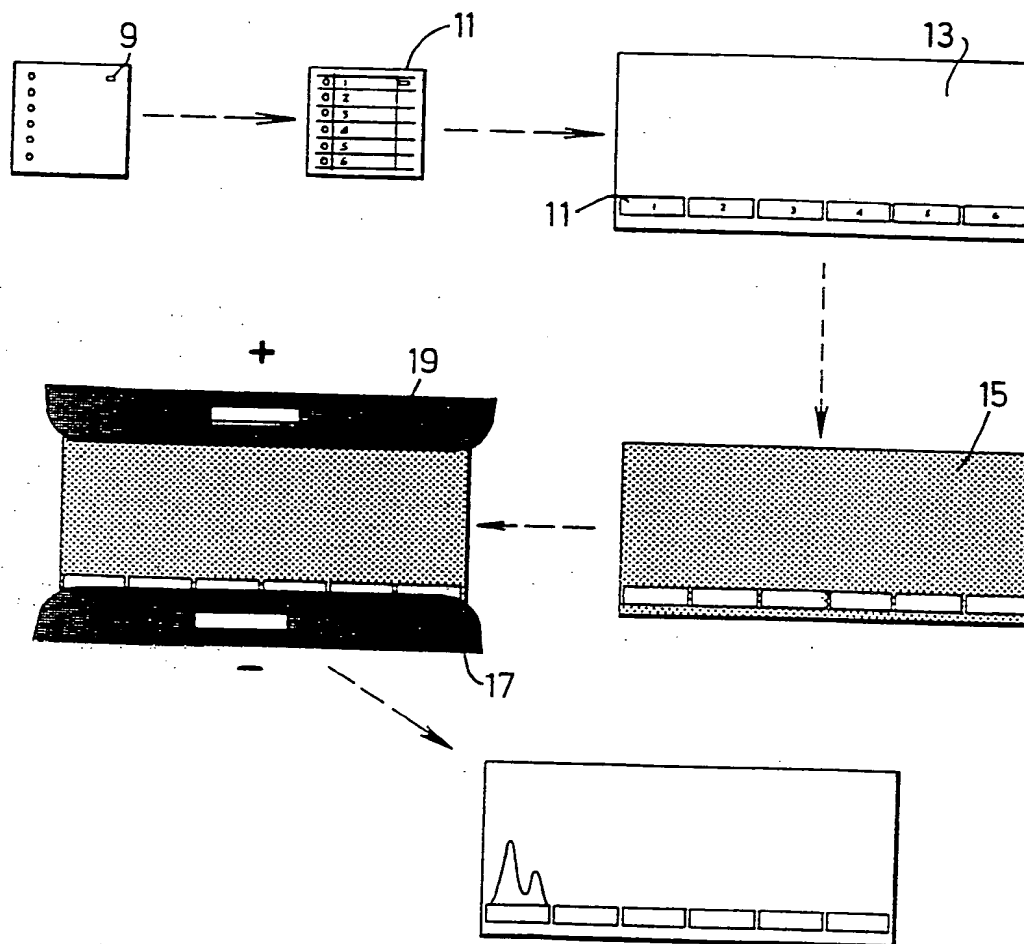
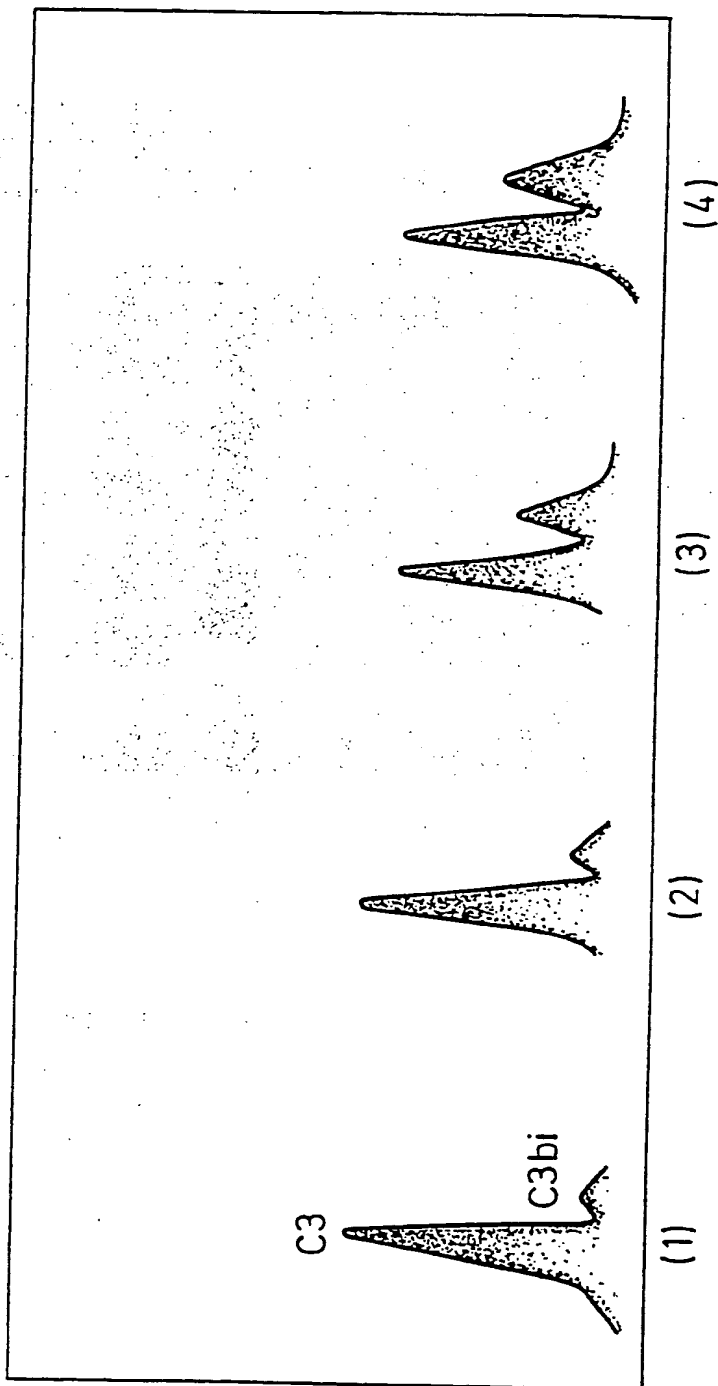


FIG. 4



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FIG. 5



6/12

FIG. 7

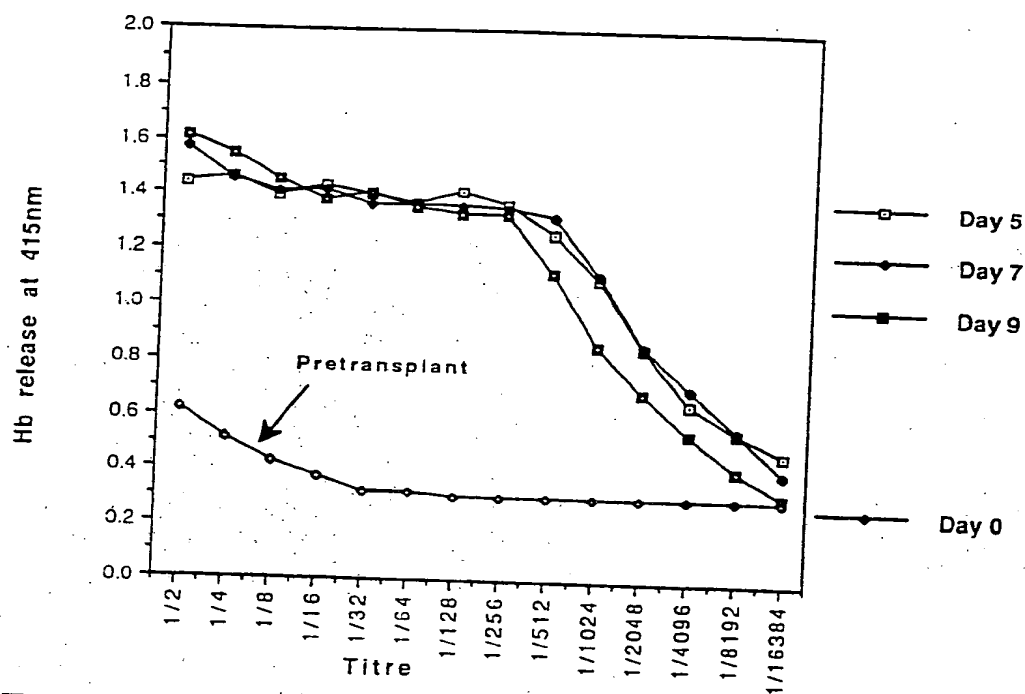
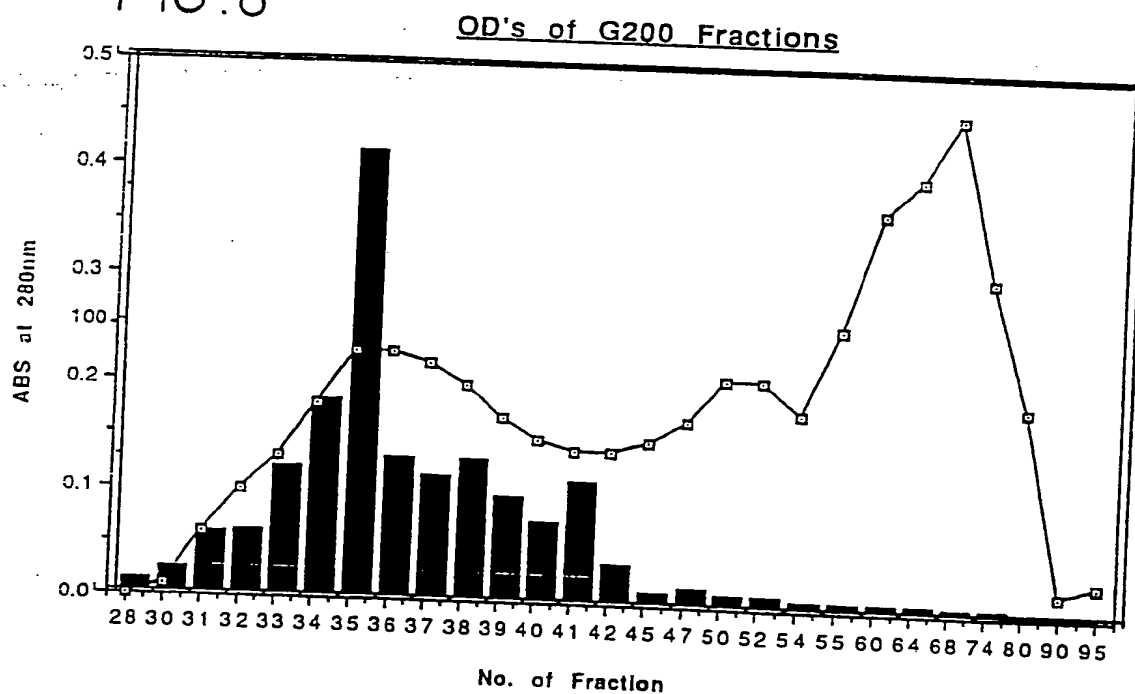


FIG. 8



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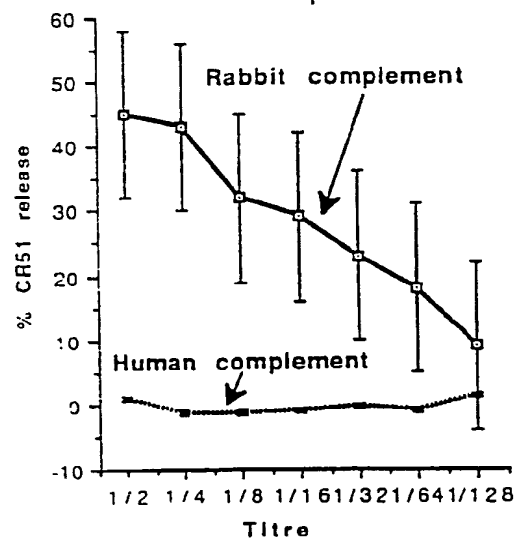
7/12



FIG. 9

T5 human cell line pig anti human
antibodies and human or rabbit
complement

FIG. 10



SUBSTITUTE SHEET

8/12

FIG. 11 Human antibodies on human cell line with rabbit or human complement

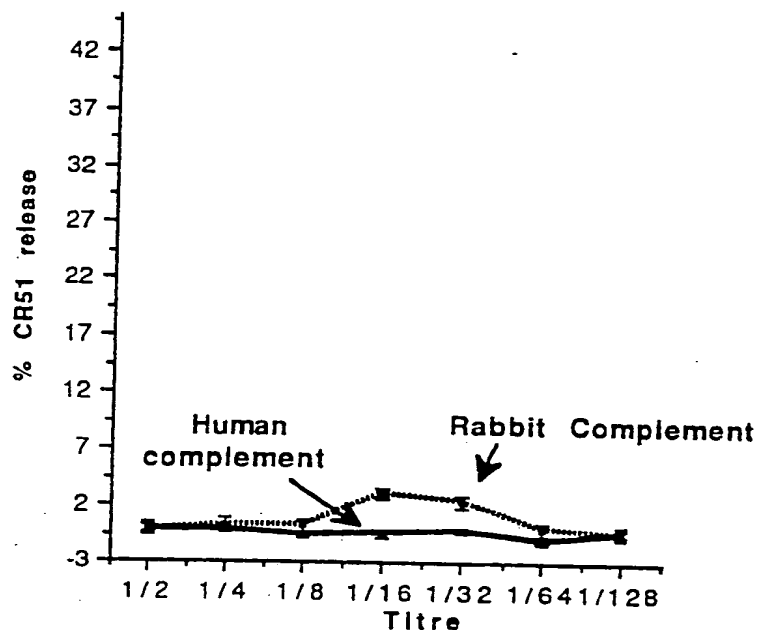
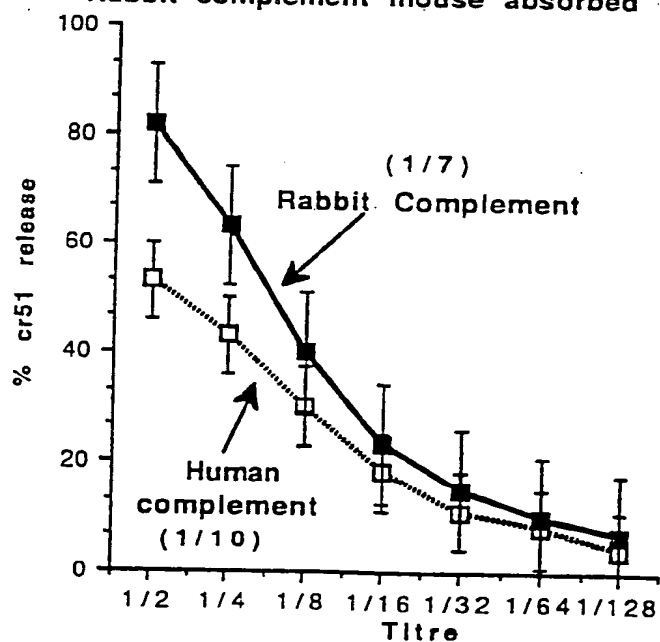


FIG. 12 Cr 51 release human anti mouse of db3 mouse/mouse hybridoma .Human and Rabbit complement mouse absorbed



SUBSTITUTE SHEET

9/12

FIG. 13 Human anti mouse antibodies killing B10 a human/mouse hybrid DAF + comparison of human & rabbit complement (absorbed)

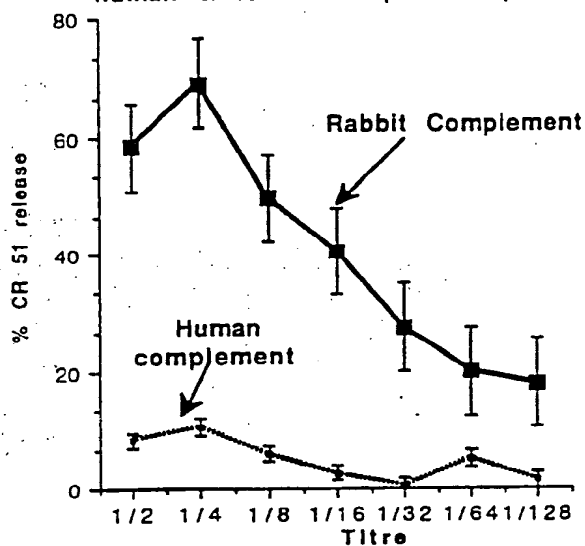
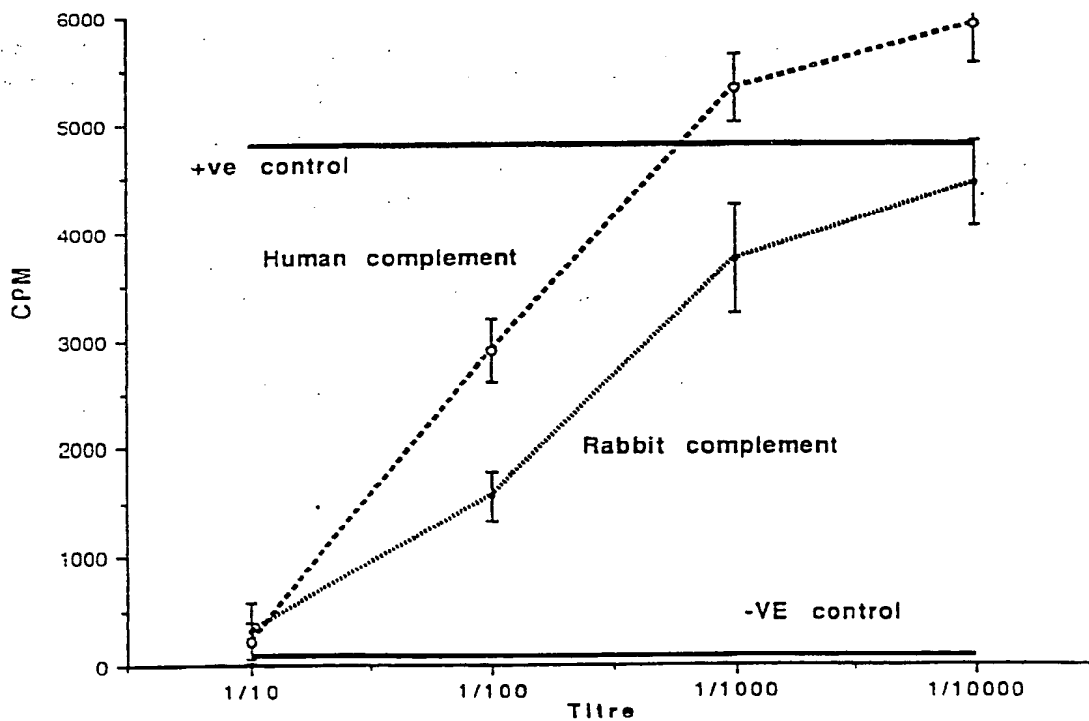


FIG. 14 Killing of CHO cells by immune rat serum

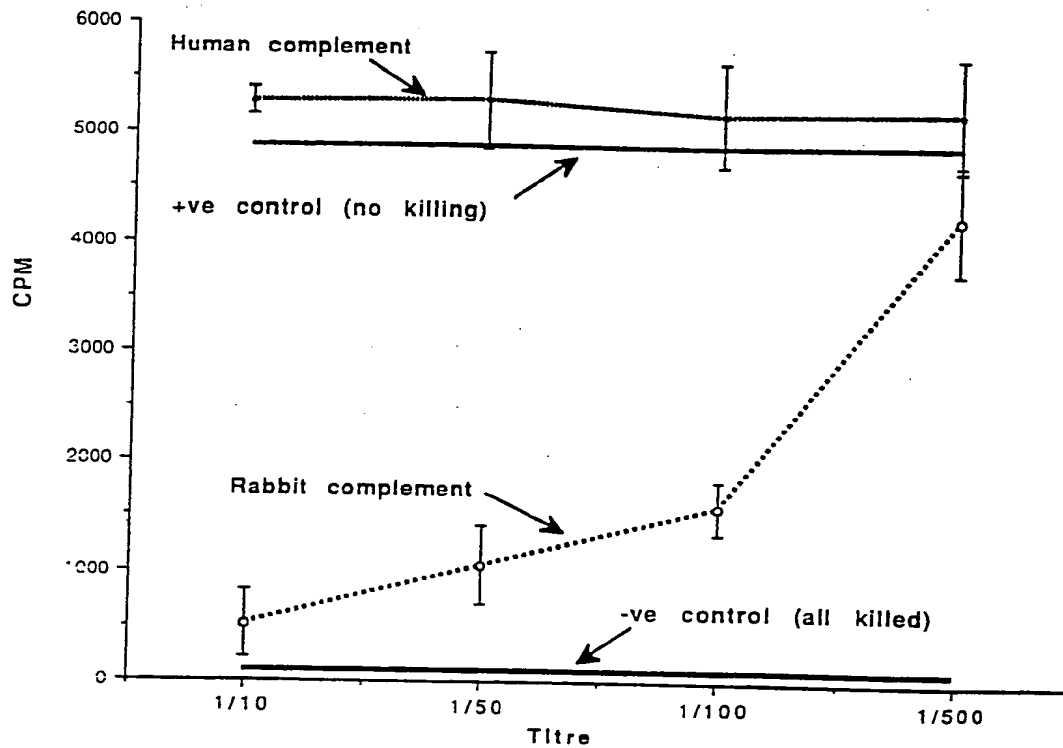


SUBSTITUTE SHEET

10/12

FIG. 15

CHO-MCP cell killing rat anti hamster
antibody rabbit or human complement 1/8



SUBSTITUTE SHEET

11/12

FIG. 16
T3 MCP+Human Cⁱ

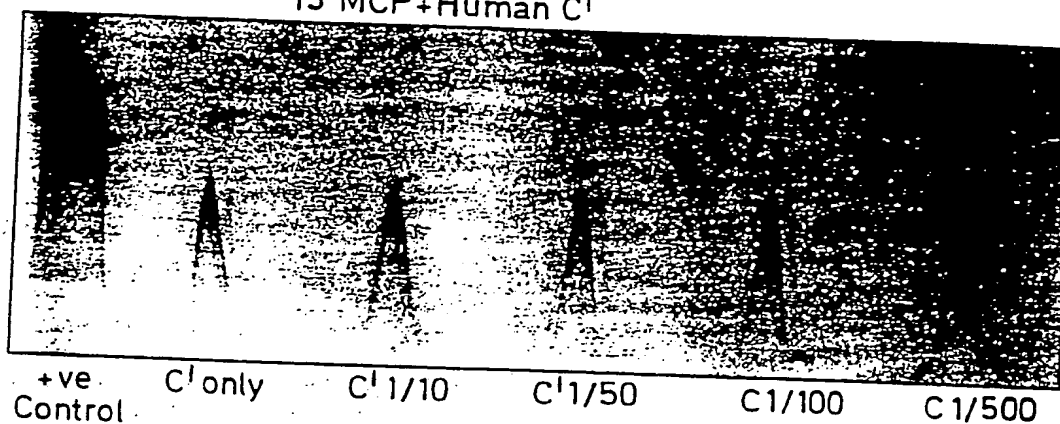
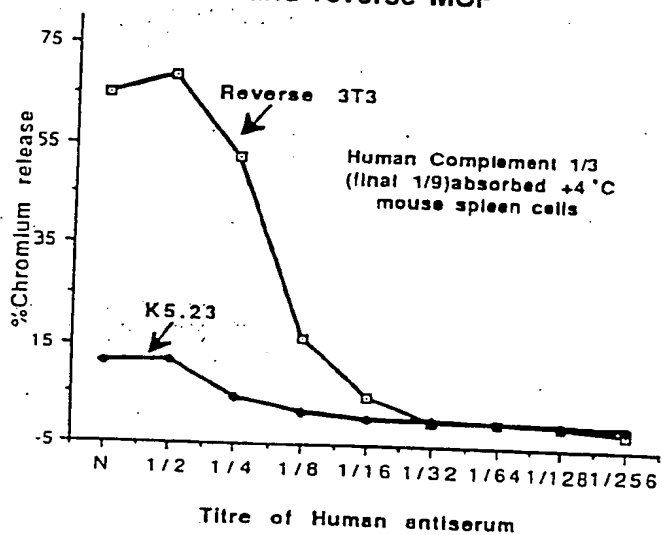


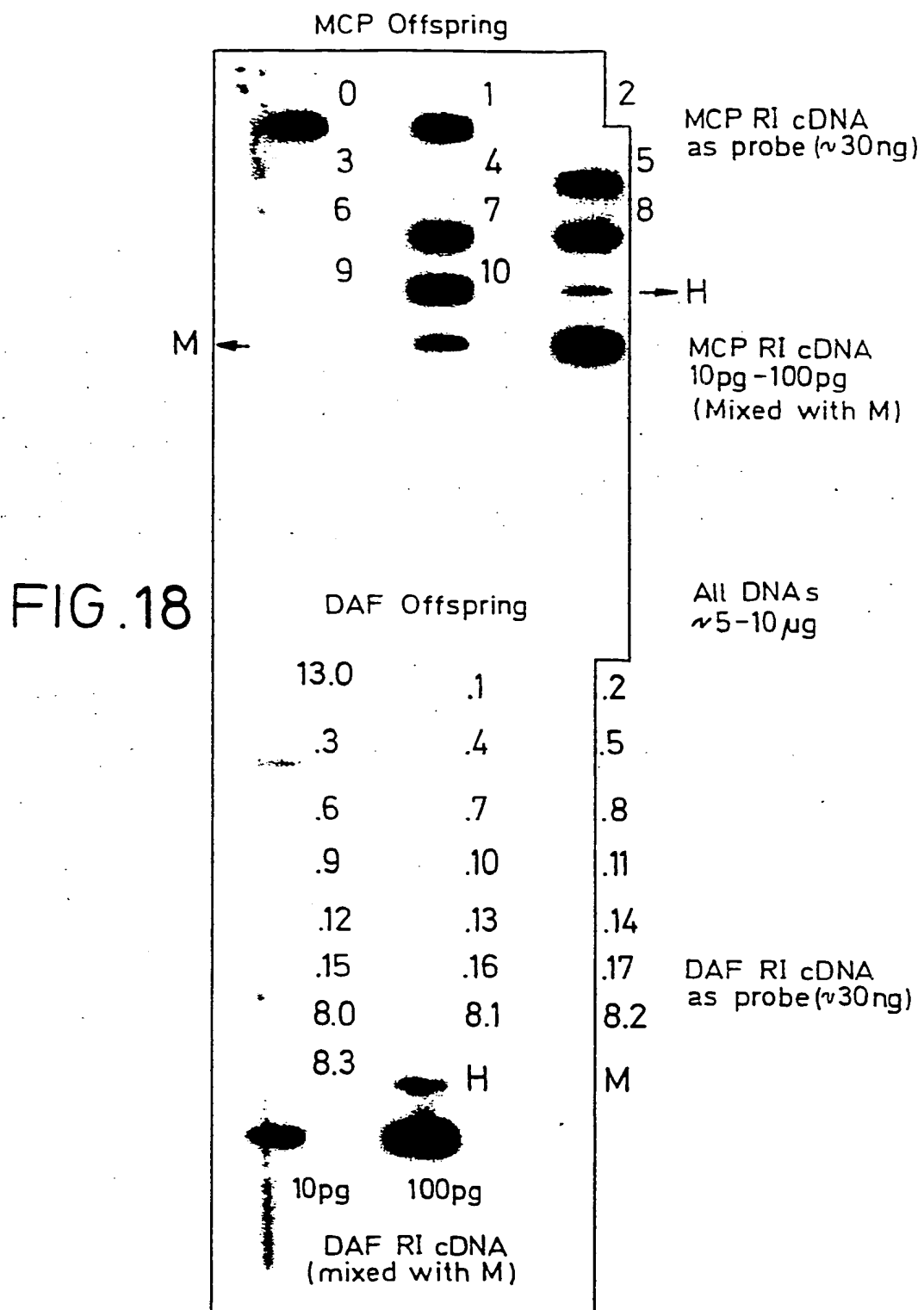
FIG. 17

Naturally occurring antibody killing of
mouse fibroblasts transfected with
MCP and reverse MCP



SUBSTITUTE SHEET

12/12



SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01575

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC ⁵ : C 12 N 5/10, C 12 N 15/12		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC ⁵	C 12 N, C 07 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ¹⁰	Citation of Document, ¹¹ with Indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	WO, A, 89/01041 (GENENTECH INC.) 9 February 1989 see pages 3-6, summary; page 27, lines 10-20; pages 32-37, examples 1,2; pages 42-43, claims --	22-24
X	WO, A, 89/09220 (THE JOHNS HOPKINS UNIVERSITY) 5 October 1989 see pages 109-121; pages 124-135, claims --	22-24
X	Journal of Experimental Medicine, vol. 168, July 1988, The Rockefeller University Press, D.M. Lublin et al.: "Molecular cloning and chromosomal localization of human membrane cofactor protein (MCP)", pages 181-194 see the whole article cited in the application -- ./.	22-24
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
26th January 1991	14. 02. 91	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	<div style="display: flex; align-items: center;"> <div style="border: 1px solid black; padding: 2px; margin-right: 10px;">E.P.O.</div> <div style="font-family: cursive; font-size: 1.2em;">M. Per3</div> </div>	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	EP, A, 0244267 (GENENTECH INC.) 4 November 1987 see the whole document; especially claims cited in the application --	22-24
A	Transplantation Proceedings, vol. 21, no. 1, February 1989, S. Miyagawa et al.: "The mechanism of discordant xenograft rejection", pages 520-521 see the whole article cited in the application --	17-27
A	EP, A, 0222661 (SANKYO CO. LTD) 20 May 1987 see the whole document --	17-27
A	Chemical Abstracts, vol. 102, no. 7, 18 February 1985, (Columbus, Ohio, US), M.E. Medof et al.: "Inhibition of complement activation on the surface of cells after incorporation of decay- accelerating factor (DAF) into their membranes", see page 455, abstract 60491v, & J. Exp. Med. 1984, 160(5), 1558-78 -----	17-27

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 1-16 because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1 (iv) - PCT :

Method for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9001575

SA 40976

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 05/02/91. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8901041	09-02-89	AU-A- 2308788 EP-A- 0371999	01-03-89 13-06-90
WO-A- 8909220	05-10-89	AU-A- 3539489	16-10-89
EP-A- 0244267	04-11-87	AU-A- 7242687 JP-A- 63102699	19-11-87 07-05-88
EP-A- 0222661	20-05-87	JP-A- 62104594	15-05-87